David D. Eisenstat · Dan Goldowitz ·
Tim F. Oberlander · Jerome Y. Yager *Editors*

Neurodevelopmental Pediatrics

Genetic and Environmental Influences

Springer

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Preface

Long before COVID-19, NeuroDevNet, a Canadian multidisciplinary network of clinicians, scientists, and trainees, recognized an unmet need in the neurodevelopmental field, where common clinical problems in Pediatric Neurology and Developmental Pediatrics needed to be thematically linked with current concepts and translational research advances that applied evidence-based interventions harnessing advances in Developmental Neurosciences, Medical Genetics, Allied Health, and related disciplines. Addressing this need would contribute to improving the lives of children and families living with brain-based developmental disorders.

In the pages that follow, we focus on advancing our understanding of the impact of genetics, environment, or both using three specific pediatric developmental disorders as exemplars of key neurodevelopmental conditions. Using autism/autism spectrum disorders (ASD; predominantly an underlying genetic predisposition), fetal alcohol spectrum disorder (FASD; predominantly an underlying environmental etiology), and cerebral palsy (CP; with combined genetic and environmental contributions), we discuss neurodevelopment spanning from molecular, behavioral, and contextual/family considerations.

The first section of the textbook provides a comprehensive and up-to-date overview of the basic science of development of the brain, including sections on stem cells, epigenetics, and the influence of the perinatal environment. This is followed by sections 2–4 where the broader scope of the three selected developmental disabilities is discussed, including the impact of genetic, epigenetic, and environmental contexts. Against the backdrop of categorical approaches to neurodevelopmental disabilities, a discussion of non-categorical dimensional approaches to assess and understand child and family health and well-being is also presented. Then chapters follow that discuss key comorbidities common to all three disorders, such as disturbed sleep, seizures, behavioral disorders, and pain. This is followed by chapters covering the impact of ASD, FAS, and CP on family dynamics, their economic impact, and service delivery to the community. Finally, important resources are provided on harnessing data as well as knowledge translation and dissemination to the community and other stakeholders. Many chapters offer a discussion of controversies and research directions as well as clinical perspectives or vignettes that provide a comprehensive approach to neurodevelopmental disabilities that will be of great interest to trainees, clinicians, and researchers alike. Our target readership are individuals with interests in the developing brain and how perturbations of that development can lead to neurodevelopmental disabilities. The aim of the text is to provide clinical and basic science trainees (residents, clinical fellows, graduate students, postdoctoral fellows) with a strong grounding in understanding disorders of the developing brain with a view to these trainees becoming the next generation of child neurologists, developmental pediatricians, and developmental neuroscientists.

As co-editors, we are indebted to our contributing authors who have devoted significant time and effort to ensure that this textbook meets the educational and biomedical needs of all

stakeholders in the neurodevelopmental community. Finally, we extend our sincere appreciation to our publishers from Springer Nature, especially Pinky Sathishkumar, who ensured that this new textbook became a reality.

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We further wish to acknowledge a host of funding agencies which have supported research specifically related to work done by the Editors inclusive of the Canadian Institutes of Health Research (CIHR), the Canadian Foundation for Innovation, the Heart and Stroke Foundation of Canada, the Natural Sciences and Engineering Council of Canada (NSERC), the Women's and Children's Health Research Institute at the University of Alberta (WCHRI), the BC Children's Hospital Research Institute, the Kids with Cancer Society (Edmonton), and the Government of Canada's Canada Research Chairs program.

The Editors would also like to thank the clinical and research trainees in Pediatrics, Pediatric Neurology, Developmental Pediatrics, Medical Genetics and Developmental Neuroscience at the University of British Columbia and University of Alberta, as well as both NeuroDevNet and the KBHN, for providing the inspiration for the textbook.

The Editors wish to express their sincere gratitude and appreciation to all of the chapter authors and co-authors of this textbook. The chapters provide an international perspective on the normal and abnormal developing child's brain. Moreover, the editors have appreciated the patience of all concerned during this trying time of the COVID-19 pandemic with all of the challenges it has brought.

Finally, the Editors are grateful to their families for their generous encouragement and support throughout the journey, from the textbook's inception to its launch.

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Part I

Overview of CNS Development and Disorders

Overview of CNS Organization and Development

1

Richard E. Brown

Learning Objectives

1. To understand the basic parameters of brain development
2. To understand the nature of the chemical signals in the brain
3. To understand the development of synapses and neural circuits in the brain
4. To understand the development of the neuroendocrine and neuroimmune systems
5. To understand the importance of critical and sensitive periods in development

Highlights

1. The importance of the interactions between chemical signals in the timing of brain development
2. The role of the endocrine and immune systems in brain development
3. The examination of how dysfunctions in synapses and neural circuits result in neurodevelopmental disorders
4. Highlights the importance of critical periods in neural development
5. Examines the epigenetic factors in brain development

Introduction: Brain Development

This chapter provides an overview of the basics of brain development, describing brain mapping, neurons, glia, synapses, and neural circuits [1]. The signaling molecules, such as neurotrophic factors, neurotransmitters, neuropeptides and peptide hormones, steroid and thyroid hormones, the transmitters, chemokines and cytokines, and the gliotransmitters are described. Signaling molecules act as epigenetic transducers, converting environmental signals to gene expression and protein synthesis, thus allowing the external and internal environment to shape neural development. Finally, neural development occurs in defined critical (or sensitive) organizational periods during prenatal and perinatal development and again during puberty, a critical period for neural activation and reorganization. During these critical organizational periods, the brain can be shaped not only by environmental input, but is also vulnerable to the disrupting influences of environmental pathogens, bacteria, viruses, and neurotoxins, including neuroendocrine disruptors which can cause abnormalities in neural development. The purpose of this chapter, therefore, is to give an overview of the building blocks of the brain which provide the background information for how abnormal neural development might underlie neurodevelopmental disorders. Greater details on specific elements of brain development are provided by Breedlove [2], Rao and Jacobson [3] and Stiles [4] and the rest of the chapters in this section.

Brain development occurs throughout the lifespan. Between conception and early adulthood, we speak of neurodevelopment and in aging we speak of neurodegeneration, but the basic elements are the same: neurons, glial cells, synapses, neural circuits, and chemical messengers. Although neurodevelopmental disorders are defined primarily in children, they are life-long conditions; they do not mysteriously disappear as soon as the child becomes an adult [5, 6]. Much of the work on the basic mechanisms of neurodevelopment has been done on rodents and examples from both humans and rodents will be used in this chapter. Although particular

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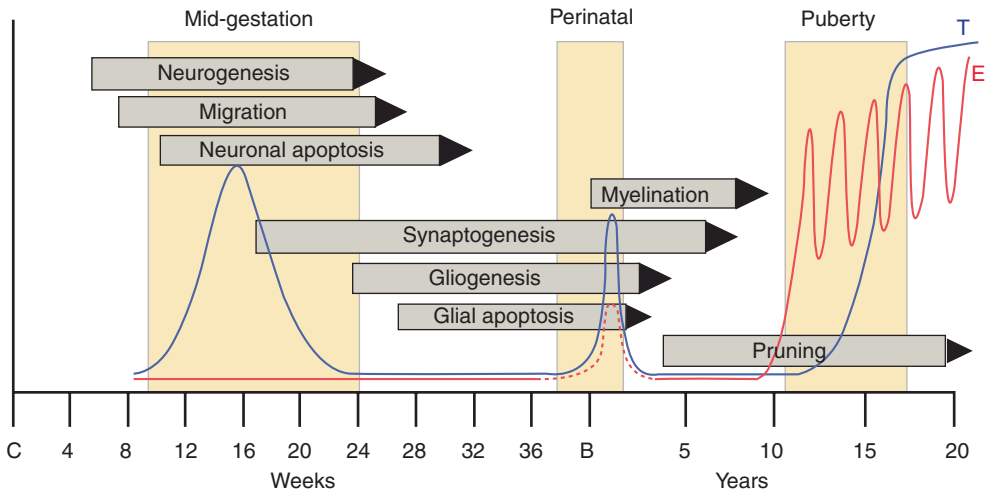

Fig. 1.1 The pattern of neural development, showing critical periods of neural organization and the timing of gonadal hormone surges. The major events in neural development are depicted during the prenatal critical period of human gestation (weeks), the perinatal critical period and the pubertal critical period (years). The gonadal hormone surges involved in sexual differentiation of the brain are shown in orange boxes. Testosterone levels in males (blue line) begin to rise during the eighth week of gestation as the testes mature, peak around week 16, and decline after week 24. Testosterone levels spike again during the perinatal critical period, with a peak around 1–3 months of postnatal age and then remain low until the onset of puberty. In females, the ovaries are largely inactive during gestation and begin to secrete estradiol (red line) during the perinatal period and during puberty. [From: Manoli DS and Tollkuhn J. 2018. Gene regulatory mechanisms underlying sex differences in brain development and psychiatric disease. Ann NY Acad Sci. 1420: 26–45. [7]]

aspects of brain development as they relate to specific neurodevelopmental disorders are discussed in the chapters of this book, the brief summary of the main features of brain development given here will introduce the neural systems underlying neurodevelopmental disorders.

The earliest steps in brain development (Fig. 1.1) are the differentiation of the embryonic neural stem cells (neural progenitor cells) in the neural plate into neurons (neurogenesis) and glial cells (gliogenesis), followed by the formation of the neural tube [5, 8, 9]. Once the neural tube has closed, the different "modules" of the brain begin to differentiate, forming the primitive telencephalon, diencephalon, and mesencephalon [1]. Brain development is orchestrated by a patterned spatiotemporal sequences of gene expression in each brain area [10]. Systematic analyses of gene expression patterns have been performed for the cortex [11], cerebellum [12], hippocampus [9], hypothalamus [13], and pituitary gland [14]. As discussed in Chap. 11, there are sex differences in gene expression during brain development (sex-biased gene expression; [10, 15, 16]) which underlie many sex differences in neurodevelopmental disorders.

Brain development goes through eight phases. These include gliogenesis and neurogenesis, the birth of glial cells and neurons; nerve cell growth, the development of axons and dendrites; the migration of neurons from the areas of the brain where they are born to their final location, and differentiation into different nerve types [5, 7, 8]. Once the nerve cells develop and migrate, neural connections are established through axon guidance mechanisms and synaptogenesis, the growth of synapses. The modification of neural connections occurs through a number of mechanisms, including nerve cell death (apoptosis), pruning of excess connections, and synaptic reorganization. External stimuli, injuries, neurotoxins and other environmental factors shape the development of the brain through epigenetic mechanisms in response to cell signaling molecules (see Sect. 1.8). Once neurons have fully developed, their axons are myelinated. As nerve cells mature and begin to synthesize and secrete neurotransmitters and neuropeptides [17–21], these early developing neurochemicals, as well as hormones, cytokines and gliotransmitters act as signals to modulate brain development. At the time of puberty, there is a second wave of neural organization, from the childhood to the adult brain, and the surge in sex hormone release activates sex differences in neural organization. All of this occurs in a spatiotemporal pattern of "critical periods" (Fig. 1.1). The same neurochemical systems are involved in age-related changes in the brain and in the neurodegenerative disorders of the aging brain [22–24], but this does not concern us here.

Types of Neurons

There are many types of neurons in the brain and CNS [25–27] and considerable effort has been made to identify each of the different types of neurons [28, 29]. Neurons can be classified according to their function (sensory neurons, motor neurons, interneurons, etc.), by their shape

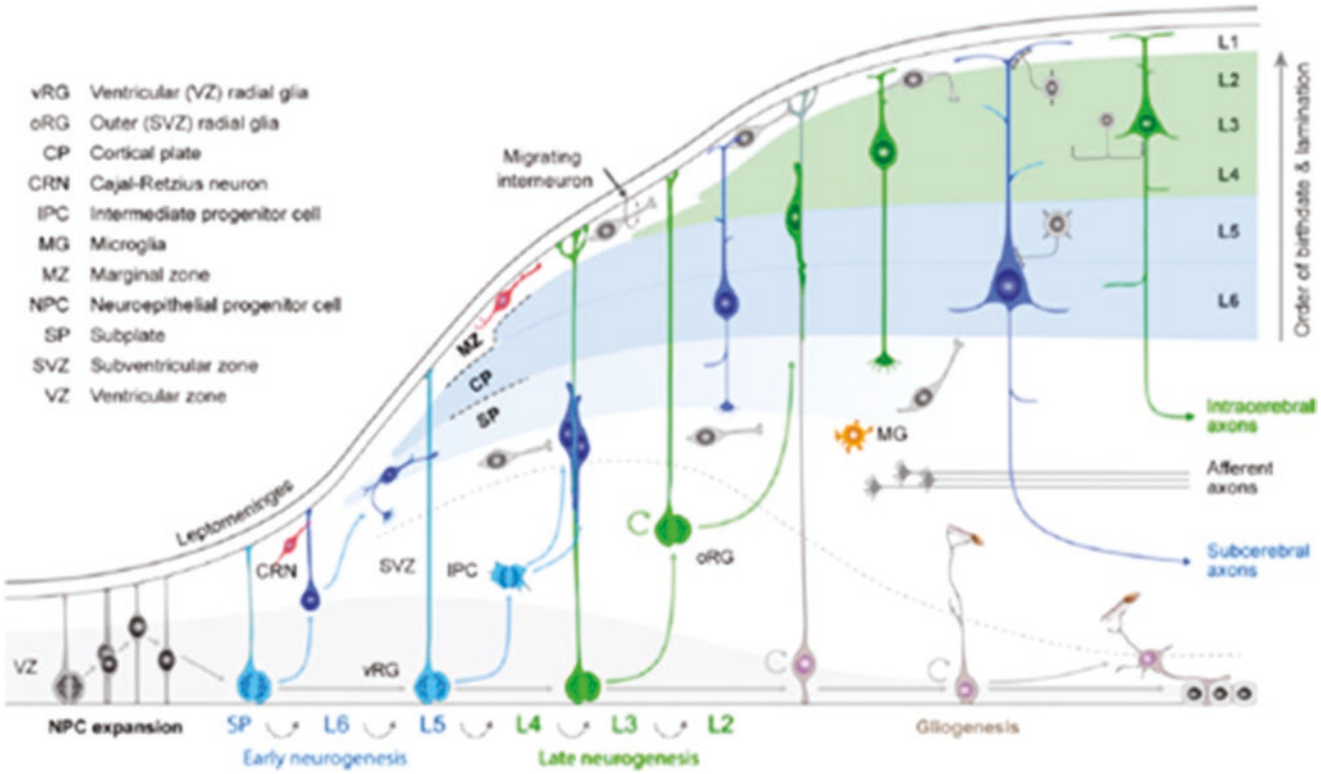

Fig. 1.2 Neuron types, astrocytes, and synapses in the development of the cerebral cortex. Neuroepithelial cells (NPCs) undergo symmetric cell division to produce. an initial pool of cortical progenitors that later transform into ventricular radial glia cells (vRGCs), which divide to generate another vRGC and a neuron. The neuron then migrates radially from the ventricular zone (VZ) along the basal process of a RGC into the cortical plate (CP). The earliest born neurons migrate to form the preplate. Later migrating neurons divide the preplate into the marginal zone (MZ) and subplate (SP). As neurogenesis proceeds, diverse subtypes of neurons are generated through the successive asymmetric division of RGCs. Early-born neurons remain in the deep layers (Layers 5 and 6; red layers), while later-born neurons settle in towards mid-neurogenesis stage. Some populations of RGC daughter cells become intermediate progenitor cells (IPCs) or outer radial glial cells (oRGCs) in the subventricular zone (SVZ). After the neurogenic stages, the radial scaffold detaches from the apical surface and vRGCs become gliogenic, generating astrocytes, or transform into ependymal cells. Tangential migration of interneurons is observed in the MZ, intermediate zone (IZ) and SVZ. Neocortical neurons mature into cortical projection neurons (CPNs), which show layer- and subtype-specific morphology and axonal projection patterns. [From: Molnar Z, Clowry GJ, Sestan N, Alzu'bi A, Bakken T, Hevner RF, Huppi PS, Kostovic I, Rakic P, Anton ES, Edwards D, Garcez P, Hoerder-Suabedissen A, Kriegstein A. 2019. New insights into the development of the human cerebral cortex. J Anat. 235: 432–451 [33]]

(monopolar neurons, bipolar neurons), by their primary neurotransmitters (dopaminergic neurons, cholinergic neurons, etc.), or by their morphology (basket cells, Purkinje cells, pyramidal cells, etc.). Neurons can also be defined by their molecular signature, as different neurons express different genes (Snap25, Gad1, Pvalb, etc.). One solution to the neuron classification conundrum is to use a hierarchical classification system based on a combination of structural, functional and molecular criteria [29, 30]. Understanding the differences between neuron types is essential for understanding the development of neural circuits and synaptic connections and determining how these patterns of development are disrupted in neurodevelopmental disorders (Fig. 1.2) [31–33].

Neurochemical Signaling Molecules and Their Receptors in Brain Development

All of the neurodevelopmental processes discussed above are genetically determined, but genes themselves do not directly regulate neural development. Gene expression is regulated by transcription factors (DNA binding factors) at DNA transcription sites [34]. Transcription factors are proteins that control the rate of transcription of DNA to mRNA and thus regulate gene expression to control each phase of neural development from neurogenesis to synaptogenesis, cell migration, and differentiation, and to regulate cell division, growth, and death [35]. The activation of many transcription factors is under the guidance of extracellular

neurochemical signaling molecules which guide brain growth and development by binding to specific receptors on their target cells to activate biochemical processes that stimulate gene transcription to produce mRNA and the translation of mRNA for protein synthesis. The genetic information in the DNA is transcribed to mRNA and is translated from the mRNA to direct the synthesis of proteins which regulate development.

Each signaling molecule must bind to its specific receptor in order to direct metabolic processes in the target cell. As the brain develops, the synthesis and release of signaling molecules must be coordinated with the production of their receptor molecules. When the neurotransmitters, neuropeptides, peptide hormones, neurotrophic factors, chemokines, and cytokines bind to their cell surface receptors, they activate a series of intracellular second messengers which form a biochemical cascade, culminating in the activation of gene transcription factors, mRNA production, and protein synthesis [36–39].

Neurotransmitters bind to two types of cell surface receptors: Ligand-gated ion channels and G-protein-coupled receptors. G-protein coupled receptors play important roles in development and aging [40, 41], and they are the targets for not only many drugs [42, 43], but they are also the targets for many neurotoxins [44]. Neurotrophins, cytokines, and chemokines bind to a number of different cell surface receptors. Neuropeptides and peptide hormones bind to G-protein-coupled receptors, while steroid and thyroid hormones, which are small molecules, can diffuse through the cell membrane and bind to intracellular nuclear receptors which act as transcription factors [45–48]. Steroid hormones can also bind to cell surface receptors to exert rapid non-genomic effects on cells [49, 50]. When neurotransmitters bind to their receptors, they activate a cascade of intracellular biochemical messengers which result in protein synthesis and cell growth.

Thus, the signaling molecules regulate the actions of neurons throughout the lifespan.

Neurotransmitters, neuropeptides and peptide hormones exert both rapid non-genomic effects at ligand-gated ion channel receptors and slow genomic effects when they bind to GPCRs, activate second messenger systems, and initiate transcription and translation of genetic information to promote protein synthesis. On a neuron, membrane receptors for neurotransmitters initiate rapid electrical changes in the cell (EPSPs or IPSPs) and the activation of transcription factors results in slower protein synthesis, cell growth, and differentiation. Steroid hormones binding to intracellular nuclear receptors (transcription factors) exert slow genomic actions in the cell, but when they bind to cell surface receptors (mostly GPCR), they can exert rapid non-genomic, neurotransmitter-like actions [39]. Signaling molecules control each stage of brain development and any neurodevelopmental or neurodegenerative disorders involve abnormal functioning of these extracellular signaling molecules or their intracellular biochemical cascades [1, 22, 23]. These are the driving forces underlying neural growth, development, differentiation and function: extracellular signaling molecules activate receptors on their target cells and these receptors control the communication between neurons and activate a chain of biochemical activity culminating in gene expression and protein synthesis.

Neurotrophic Factors

Neurotrophic factors are proteins/peptides which promote nerve cell survival, growth and differentiation, axon and dendrite growth, and axon guidance. They include Nerve Growth Factor (NGF), Neurotrophin 3 (NT-3), Brain-Derived Neurotrophic Factor (BDNF), Glial-Derived Neurotrophic Factor (GDNF), Fibroblast Growth Factor (FGF), and Insulin-like Growth Factors (IGFs). Neurotrophins bind to specific Tyrosine kinase (Trk) and p75NTR cell surface receptors which activate intracellular signaling cascades to activate transcription factors (Sp1, NF1, NFkB, CREB, FOXP1, AP-1) and initiate gene expression in their target cells [34, 35, 51–53]. Neurotrophins are essential for the development of the brain and nervous system [54–56]. They are also essential signaling molecules for learning and memory [57, 58]. Disruption of neurotrophic signaling during development results in disorders of neural organization and function, leading to neurodevelopmental and neurocognitive disorders such as Autism, Fragile X disorder, Rett syndrome, and ADHD [57, 59, 60].

Neurotransmitters

The "classic" neurotransmitters (NT): Acetylcholine (Ach), Noradrenaline (NE), Dopamine (DA), Serotonin (5-HT), Glutamate (Glu), and GABA bind to their receptors on the cell surface to activate intracellular signaling cascades. These receptors can be ligand-gated ion channels or GPCR [39, 57, 61]. When neurotransmitters bind to ligand-gated ion channels, they cause rapid changes in membrane depolarization, initiating EPSPs or IPSPs. When NT bind to GPCR, they activate cascades of intracellular second messengers to activate transcription factors and protein synthesis [43]. Neurotransmitters are the primary signaling molecules at synapses and any disruption of neurotransmitter signaling results in neurodevelopmental, cognitive, emotional, and neurodegenerative disorders [19, 57, 62]. Neurotransmitter action at GPCR is an essential mechanism for neural development [18, 41, 63]. GPCR are the targets of not only many drugs [42, 43] but they are also the targets for many neurotoxins [44].

Neuropeptides and Peptide Hormones

The neuropeptides and peptide hormones, such as Substance P, the Internal Opioids, Neuropeptide Y, Galanin, Somatostatin and others bind to their GPCR and activate intracellular second messenger systems, leading to protein synthesis [39]. Like neurotransmitters, neuropeptides can activate EPSPs in post-synaptic cells and form neural circuits [64]. Neuropeptides play an important role in neural development [65] and have been implicated in neurodevelopmental disorders [66].

Steroid and Thyroid Hormones

The steroid and thyroid hormones are small enough to go through the cell membrane and bind to intracellular receptors that act as nuclear transcription factors to regulate growth and differentiation of their target cells [49, 67–69]. The estrogen receptors (ERa and ERb), progesterone receptors (PR), androgen receptors (AR) and the glucocorticoid receptor (GR), as well as the thyroid hormone receptors all have significant effects on the development of the brain and body [70–72]. As well as binding to classic intracellular receptors, steroid hormones can also bind to GPCR to initiate rapid non-genomic neurotransmitter-like actions [50]. The sex steroid hormones are essential for sexual differentiation of the brain and body during the perinatal and pubertal organizational periods [73, 74]. Steroid hormones are also synthesized in the brain as neurosteroids [75]. A number of environmental chemicals, termed "endocrine disruptors" can interfere with the action of steroid, thyroid and other hormones at their receptors and can disrupt neural and physical development (See Sect. 1.6), particularly with regard to sexual differentiation [76–78].

Chemokines and Cytokines

Chemokines and cytokines are the signaling molecules of the immune system. They bind to Tyrosine kinase and p75 receptors at their target cells to initiate immune and inflammatory responses [79, 80]. The cytokines (interleukins, interferons, Tumor necrosis factors) and chemokines are primarily involved not only in neuroinflammation but also regulate a number of neurodevelopmental and neurodegenerative disorders [80–82]. Cytokines are also involved in neural development and in the sexual differentiation of the brain [83–85].

Synapses and Neural circuits

Synapses

The synapse is the basic unit of communication throughout the brain. Without synapses, neurons could not communicate with one another to form neural circuits. The synapse, as defined by Sir Charles Sherrington in 1897, is the junction between two neurons, the pre-synaptic and post-synaptic cells (see [86]). However, there are many different types of synapses [87]. Synapses can be electrical (gap junctions; [88]) or chemical, and chemical synapses can be excitatory or inhibitory [89]. Synapses can also be defined by their primary neurotransmitters, as in cholinergic or adrenergic synapses, or by their location as in neuro-muscular synapses. Within the brain, two neurons can have axo-dendritic, axo-axonic, axo-somatic, or dendro-dendritic synapses. Each synapse is a complex structure. The release and reuptake of neurotransmitters from a pre-synaptic neuron involves a number of biochemical processes as does the activation of a post-synaptic neuron when the neurotransmitter binds to its receptor [90]. Indeed, Zhu et al., [91] defined 37 different types of synapse in the mouse brain. To further complicate matters, there may be more than one neurotransmitter released at a synapse: often a "classical" neurotransmitter and one or more neuropeptides as "cotransmitters" [92–95]. Thus, the synaptic connections between neurons can be very complex (see below for the role of glial cells).

Synaptogenesis is the formation of new synapses and there is a continuous change in the number of synapses, with new synapses being formed, pruned and eliminated constantly, thus the term "the dynamic synapse" [96]. Synapses differ in size and strength, can be altered quite rapidly, and are sensitive to modulation by all manner of neurochemicals [97]. The synaptome is the map of all of the synapses in the brain and the synaptome shows continuous change over the lifespan [98]. Finally, as will be discussed below, synaptogenesis, synaptic pruning, and synapse elimination are critical for shaping brain development in response to internal and external stimuli during critical (sensitive) periods of neural circuit development [99]. Activity-dependent synaptic plasticity occurs during neural development and forms the basis of perceptual processes, learning, and memory [100–102]. Disorders of synaptic plasticity underlie many neurodevelopmental disorders [62, 90, 103, 104]. There are many ways that synapses can go wrong in NDD and Grant [105] has developed a synaptome theory of neural disorders.

Neural Circuits

Synapses allow neurons to form neural circuits and the "connectome" defines a neural network or functional neural pathway for the processing of information and the control of behavior [106]. Thus, a neural circuit or cell assembly is a set of neurons connected via excitatory and inhibitory synapses to form a functional unit or neural network. In order to understand the pattern of neural connections in the brain (the connectome), one must understand the patterns of synaptic connections between cells [107]. There are four basic types of neural circuits: diverging circuits, converging circuits, reverberating circuits, and parallel after-discharge circuits [108]. In a diverging circuit, one neuron synapses with a number of post-synaptic cells, while in a converging circuit, inputs from many sources converge into one output. In a reverberating circuit, one or more neurons in the circuit sends a signal back to initiating neuron to produce a repetitive output. In a parallel after-discharge circuit, a neuron inputs to several chains of neurons, which can result in an after-discharge; continued firing after the stimulus has stopped. There are many models of neural circuits in the brain [109, 110] and new methods for studying neural circuits and the specific neural cells involved in them continue to be developed [111].

The development of neural circuits thus depends on neural development, (neurogenesis), synapse formation (synaptogenesis) and the communication between neurons via electrical or chemical messengers [112]. Synapse formation and maintenance occur through complex genetic and biochemical mechanisms and the development of neural circuits is shaped by synaptic activity [107, 113, 114]. Abnormal development of neural circuits can be one of the causes underlying neurodevelopmental disorders [90, 115–117]. Abnormalities in specific neural circuits have been proposed to underlie autism spectrum disorder, ADHD, and schizophrenia [115, 116, 118, 119]. For example, abnormalities in dopaminergic circuits may underlie a number of NDD, including Fragile X syndrome, Angelman syndrome, and Rett syndrome [120]. Dysfunctional neural circuits involving glutamate or GABA may underlie other NDD [121, 122]. Connectome or neural network maps are important for understanding gene expression and the formation of synapses in neural pathways and for understanding the abnormalities in brain communication networks underlying neurodevelopmental disorders (Fig. 1.3) [123, 124]. An advantage of the neural network approach for studying neurodevelopmental disorders is that it provides a whole-brain approach to the study of brain activity [125].

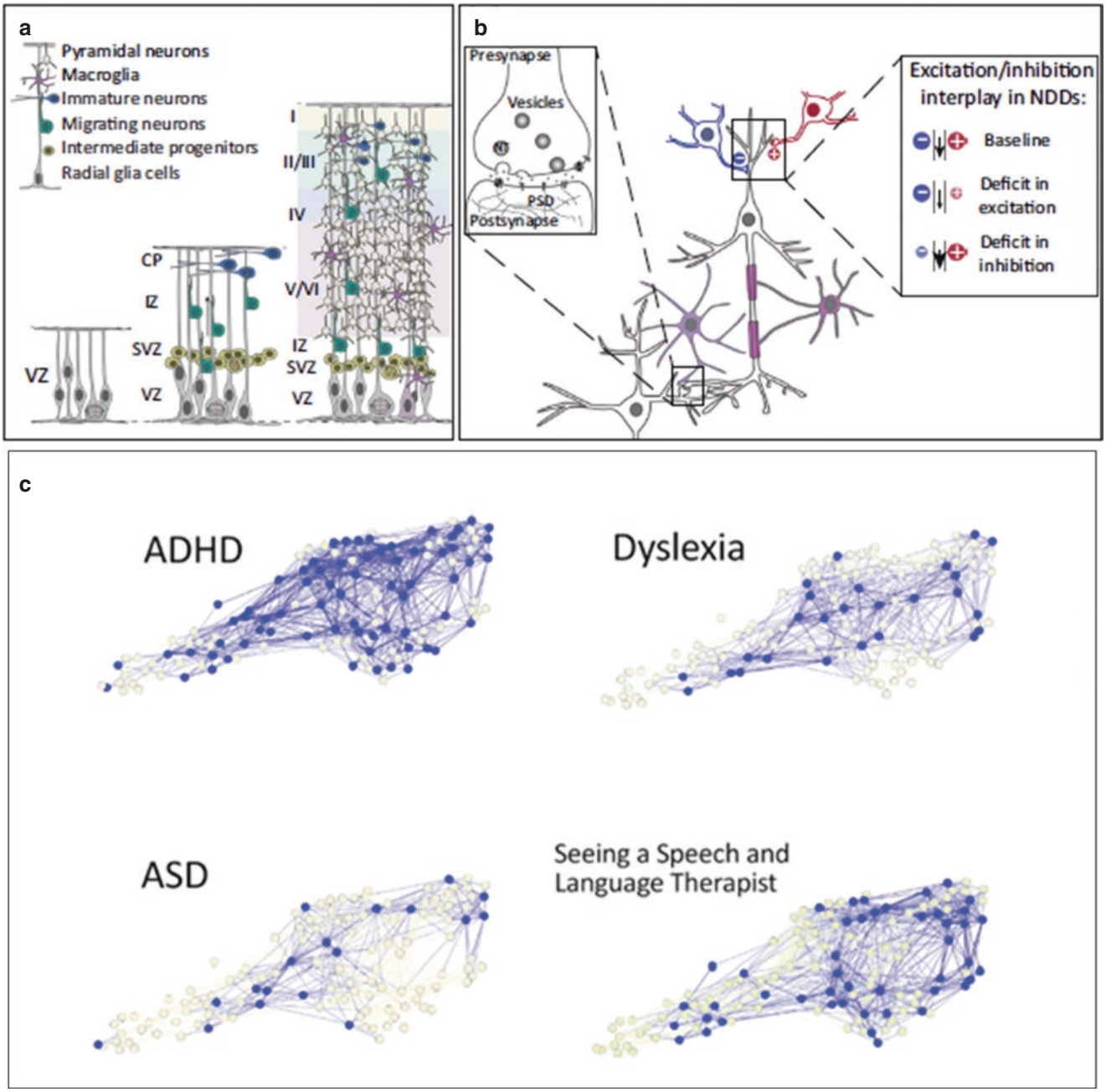

Fig. 1.3 Neural circuits underlying neurodevelopmental disorders. Genes associated with NDD are involved in the regulation of protein synthesis, transcriptional and epigenetic regulation; synaptic maturation and synaptic signaling. (**a**) Abnormalities in these processes can occur during neurogenesis, migration of neuron migration, and differentiation during prenatal brain development, or (**b**) alter synaptic maturation and proper emergence of inhibitory/excitatory balance in postnatal development. The different NDD-associated genes affect the development of particular molecular pathways and processes which regulate excitatory/inhibitory balance. Abbreviations: *AP* Action potential, CP cortical plate, *IZ* inner subventricular zone, *PSD* post-synaptic density, *SVZ* subventricular zone, *VZ* ventricular zone. (From: Parenti I, Rabaneda LG, Schoen H, Novarino G. 2020. Neurodevelopmental disorders: From genetics to functional pathways. Trends in Neuroscience. 43(8):608–621 [117]]. (**c**). A whole-brain network approach to elucidating the neural networks underlying different neurodevelopmental disorders. These are the results of machine learning computer models based on data from cognitive testing, structural neuroimaging and diffusion-weighted imaging using Euclidean distance and a Force Atlas layout. The result is a whole-brain white matter connectome in which the "best matching units" (BMUs) of different diagnoses are highlighted in blue. [From: Siugzdaite R, Bathelt J, Holmes J, Astle DE. 2020. Transdiagnostic brain mapping in developmental disorders. Current Biology. 30: 1245–1257 [124]]

Glial Cells and Brain Development

There are a number of different types of glial cells in the brain (neuroglia), CNS and peripheral nervous system. Four of these are important in the neuronal function of the brain: radial glia, microglia, astrocytes, and oligodendrocytes [126]. In many ways, the glial cells orchestrate the spatio-temporal organization of the developing nervous system [127]. Fig. 1.4 shows the actions of these types of glial cells in the brain. Glial cells also play an important role in the sexual differentiation of the brain [83, 128].

Radial glial cells develop from neuroepithelial cells and function as "ladders" for neurons to migrate from their sites of synthesis in the ventricular zone to their final destinations in the brain [129, 130]. During certain "critical periods" of brain development, radial glia cells function as neural stem cells to generate neurons, astrocytes and oligodendrocytes in the CNS [127, 131, 132]. Radial glial cells are important in the generation, migration and differentiation of neurons and thus "direct" the patterning of neural development.

Microglia are critical regulators of neural development and differentiation and are involved in all of the stages of neural development (Fig. 1.1) [133–135]. Microglia are involved in the regulation of cell survival, cell proliferation and cell death. They are also involved in synaptic plasticity and the remodeling of synaptic circuits through synaptic pruning, activity-dependent modulation of synapses, and the effects of injury and neurotoxins [136–138]. Since microglia can regulate synaptic activity, abnormal microglial activity can disrupt synaptic function in neural circuits and lead to neurodevelopmental disorders [139, 140].

Astrocytes are intricately involved in synaptogenesis and synaptic activity and communicate with neurons via a number of chemical signals in "tripartite" synaptic activity [138, 141]. Astrocytes communicate between the blood and the brain through the blood–brain barrier, provide nutrients to neurons, and modulate neurotransmitter action and ion channel activity in neurons [127]. Astrocytes are also involved in synaptic pruning and other mechanisms of synapse remodeling [136]. Thus, astrocytes modulate the activity of neural circuits by regulating synaptic activity and the propagation of nerve impulses along axons [127]. By regulating synaptic development and pruning, astrocytes play an important role in the development of neural circuits [31].

The oligodendrocytes form the myelin sheath around nerve axons in the brain and thus form the white matter of

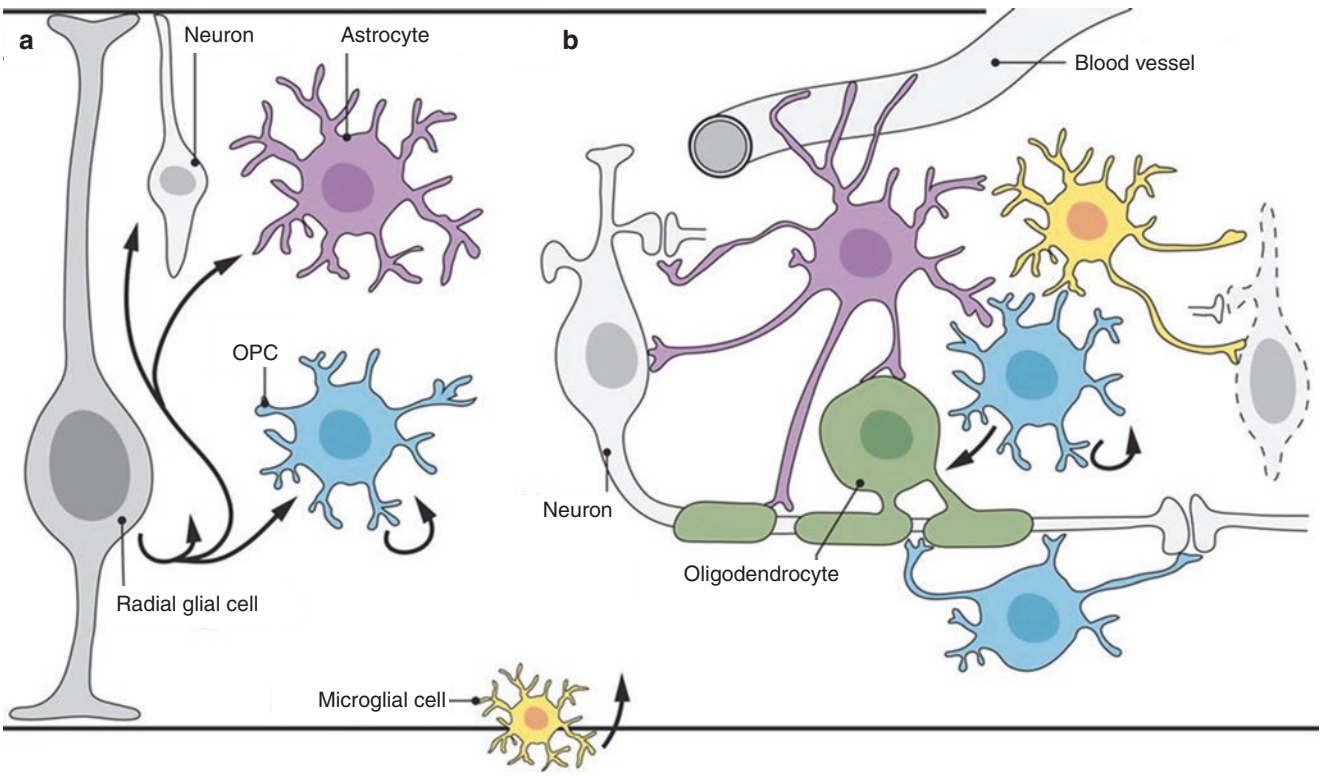

Fig. 1.4 Glial cells in the CNS. (**a**) Radial glial cells are the principal neuroepithelial progenitor cells of the central nervous system and generate the majority of CNS neurons and glia, either directly (e.g., neurons) or indirectly through intermediate progenitors (e.g., oligodendrocyte progenitor cells, OPCs). Microglia (yellow) enter the CNS during embryonic. development. (**b**) Neurons and glia interact in a myriad of different ways. The dashed line indicates a dying neuron. [Adapted From: Allen NJ and Lyons DA. [127]. Glia as architects of central nervous system formation and function. Science. 362: 181–185.]

the brain [141–143]. Myelination occurs late in neural development (Fig. 1.1) and involves a number of steps, including the proliferation and migration of oligodendrocyte precursor cells (OPCs), the recognition of target axons and axon–glia signaling, the differentiation of OPCs into myelinating oligodendrocytes which then wrap around the axons. This is followed by myelin compaction, and the formation of the nodes of Ranvier [143]. Throughout the process of myelination, there is chemical signaling between the neurons and oligodendrocytes. Once the myelin sheath is complete, the oligodendrocytes provide insulation and trophic support to neurons and regulate the structural and electrical properties of the axons they myelinate [141].

The Tripartite Synapse and Gliotransmitters

As noted above, glial cells play an important role in brain development and are intricately involved with synapses, forming a "Tripartite synapse" with the pre- and post-synaptic neurons [144–146]. In this way, astrocytes and microglia regulate neurogenesis, synaptogenesis, synaptic pruning, and apoptosis (cell death), during development and may be involved in the sexual differentiation of synapses and neural circuits [147]. Both astrocytes and microglia play a role in synaptic activity [138, 148, 149], thus the tripartite synapse has also been called the "Quad-partite synapse" or "tetrapartite synapse" [150, 151]). Glial cells release chemical signals which have been termed "gliotransmitters" [144]. Although primarily released from astrocytes, gliotransmitters are also released from microglia and oligodendrocytes and include classical neurotransmitters, neuromodulators, hormones, cytokines and growth factors [152].

There are a number of ways in which glial cells and neurons can communicate [153, 154]. These include the elevation in calcium levels in glial cells in response to the release of neurotransmitters such as glutamate or GABA by neurons; the release of gliotransmitters from glial cells and the response of pre- and post-synaptic neurons to the gliotransmitters. The specific gliotransmitter released may depend on the synaptic activity and the response of the neurons to the gliotransmitters may depend on the pre- or post-synaptic location of binding and type of receptors activated, as well as the neuronal cell type, so that the neuron-glial signaling may be synapse specific.

Development of the Neuroendocrine System: The Hypothalamic-Pituitary System

The Hypothalamus

The hypothalamus maintains the body's homeostatic systems: it is where the brain meets the body. Specific nuclei of the hypothalamus regulate food intake, metabolism and body weight, thirst and water balance, body temperature, blood pressure, heart rate, stress responses, aggressive, reproductive and maternal behaviour, and the regulation of hormone secretion [39, 155–158]. Hypothalamic hormones are released from the "neuroendocrine hypothalamus" and the release of these hormones is regulated by neurotransmitters and neuropeptides, whose secretion is, in turn, modulated by steroid hormones [39]. The hypothalamus regulates the secretion of hormones from the anterior pituitary gland (Adenohypophysis) through the release of a number of hypothalamic hypophyseal hormones [39]. A number of hypothalamic nuclei have neurosecretory cells that release the hypothalamic hormones into the bloodstream (primary plexus) and these hormones travel through the hypophyseal portal veins in the pars tuberalis to the anterior pituitary where they regulate the release of the pituitary hormones.

The Pituitary Gland

The pituitary gland has two distinct parts: the adenohypophysis (anterior pituitary) and the neurohypophysis (posterior pituitary), each of which is connected to the hypothalamus ([159]; Brown and [39, 160]). The neurohypophysis is actually a part of the brain. It is formed from neural tissue from the neuroectoderm of the diencephalon. The adenohypophysis is an endocrine gland formed from the ectoderm. During development, the two parts of the pituitary gland become intertwined through a complex series of genetic, epigenetic, and hormonal interactions [159, 161–167]. The neurohypophysis stores and secretes the neurohormones Oxytocin (OXY) and Arginine Vasopressin (AVP; also called Anti-Diuretic Hormone), which are synthesized in the magnocellular neurons of the Supraoptic and paraventricular nuclei of the hypothalamus. The adenohypophysis contains five types of endocrine cells in the pars distalis which secrete growth hormone, prolactin, LH and FSH, ACTH, and TRH as well as cells in the pars intermedia which secrete MSH. Synthesis and secretion of the hormones of the adenohypophysis are regulated by the hypothalamic hypophyseal hormones, which are secreted from the parvocellular neurons of the paraventricular nucleus, ventromedial nucleus and other nuclei of the hypothalamus and released into the bloodstream in the median eminence of the hypothalamus, from which they travel down the hypophyseal portal veins of the pars tuberalis in the pituitary stalk to the adenohypophysis.

The Neuroendocrine System and Neurodevelopmental Disorders

The neuroendocrine system develops in parallel with the brain [168, 169]. The neurohypophyseal hormones, oxytocin and vasopressin, are produced in the Supraoptic nucleus

(SON) and the Paraventricular nucleus (PVN) of the hypothalamus which send their axons down the hypophyseal stalk (Infundibulum) to the posterior pituitary gland where these hormones are released into the bloodstream [39, 170]. In addition to their hormonal function in the bloodstream, oxytocin and vasopressin are also released into neural pathways In the brain where they act as neuromodulators ([171–173]). Both oxytocin and vasopressin have been implicated in neurodevelopmental disorders, particularly in autism spectrum disorders [174–176].

The hypothalamus also secretes a number of hypophysiotropic hormones which can stimulate or inhibit the release of hormones from the pituitary gland as it develops [165, 177]. These anterior pituitary hormones activate the peripheral endocrine glands to secrete their hormones [39, 178–181]. As the prenatal neuroendocrine system develops [17, 21], the early acting hormones modulate neural development as shown in Fig. 1.1. There are three main hypothalamic-pituitary-hormone systems that affect brain development: the hypothalamic-pituitary-thyroid (HPT) system, the hypothalamic-pituitary-adrenal (HPA) system and the hypothalamic-pituitary-gonadal (HPG) system. Disruptions of any of these hypothalamic-pituitary neuroendocrine systems can affect neurodevelopmental disorders.

The hypothalamic thyrotrophin-releasing hormone (TRH) stimulates thyroid-stimulating hormone (thyrotrophin) from the anterior pituitary and this stimulates T3 and T4 from the thyroid gland. These thyroid hormones are secreted early in embryonic development and are crucial for neural development [45, 182]. Children with congenital hypothyroidism have a number of neurological disorders including neurodevelopmental delay, speech and hearing impediments, voluntary motor movement impairments, and can also result in severe neurocognitive impairment [72, 183]. The synthesis of thyroid hormones depends on iodine, thus a lack of iodine in the diet results in hypothyroidism [184]. Environmental chemicals that disrupt thyroid hormone synthesis also cause hypothyroidism [185].

The hypothalamic corticotrophin releasing hormone (CRH) stimulates adrenocorticotrophic hormone (ACTH) secretion from the anterior pituitary and this stimulates glucocorticoid (GC) secretion from the adrenal glands. The HPA system is activated during stress, and high levels of adrenal glucocorticoids (Adrenal steroids) inhibit neural development. Excessive secretion of corticosteroids during development results in neuropathology [186, 187], thus maternal stress or the injection of synthetic glucocorticoids, such as dexamethasone, into the mother during pregnancy may disrupt neural development in the fetus [188].

The hypothalamic Gonadotrophin-Releasing Hormone (GnRH) stimulates Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) from the anterior pituitary which then stimulate the gonads to produce the sex steroids: estrogen, progesterone and androgens. The activation of the HPG system results in the sexual differentiation of the brain and body during the perinatal organizational critical period and the pubertal organizational–activational period (see Fig. 1.1). As a result of the genes on the sex chromosomes that determine gonadal sex, the HPG systems of males and females develop differently (Chap. 11) and the gonadal hormones direct the sexual differentiation of the genitals, the brain and many other organs in the body. As a result of this sexual differentiation process, males and females show different patterns of brain activity, have different neuroendocrine systems, and have different patterns of neurodevelopmental and neurodegenerative diseases [180, 189–194].

The other hormones of the anterior pituitary, growth hormone [195–198] and prolactin [199] may also be involved in NDD.

Development of the Neuroimmune System

Like the neuroendocrine system, the neuroimmune system goes through a patterned spatiotemporal sequence of developmental stages (Fig. 1.5) from fetal life to old age [200–203]. There are two "branches" of the immune system: the innate immune system and the adaptive (acquired) immune system [204–206]. Both of these systems develop during fetal life but do not become completely active until after birth. This means that the newborn infant is susceptible to many forms of infection before the immune system is fully developed [202, 207, 208]. Some antibodies are transferred from the mother to the infant through the placenta and breast milk, but the newborn is still susceptible to a multitude of pathogens (viruses, bacteria, fungi, and parasites) until the immune system matures. This is the reason for the high level of infant mortality: their immune system takes time to develop [209]. The neonatal immune system thus goes through an important critical period in which it learns to discriminate between "safe" and "dangerous" external stimuli [210, 211].

The immune system develops in parallel with the brain and neuroendocrine system and the cytokines from immune cells interact with neural and endocrine cells during development [212]. Likewise, neurotransmitters, neuropeptides and hormones interact with the developing immune system. Like the nervous system, the immune system has the capacity for learning and memory [200, 212–215]. Immune system plays an important role in the development of the brain and nervous system (Fig. 1.8), modulating the development of both neurons and glial cells [128, 218, 219]. The steroid hormones of the HPA and HPG systems, particularly the glucocorticoids (corticosteroids) and the sex hormones (estrogen and androgens), have well-documented effects on

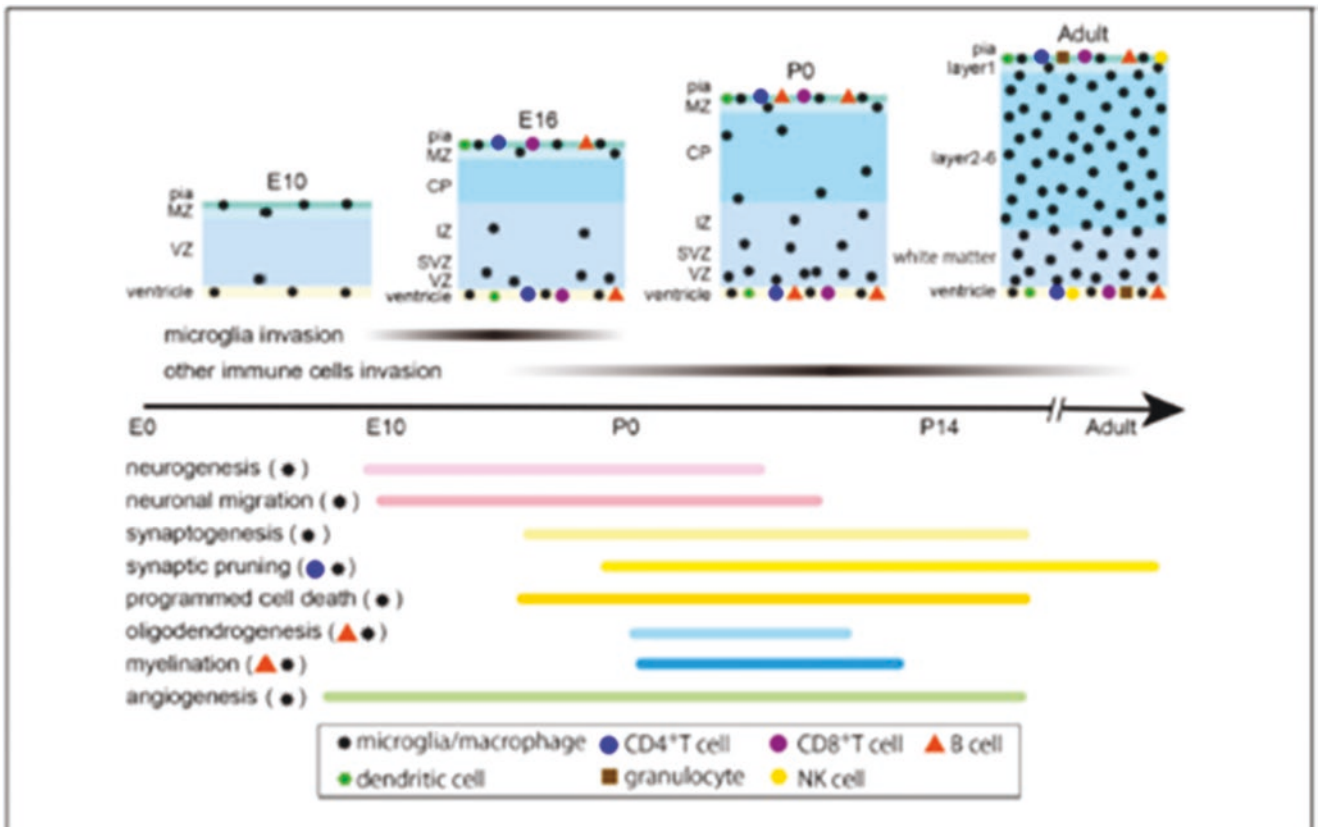

Fig. 1.5 The role of the immune system in the development of the central nervous system. A timeline showing the distribution of immune cells during cerebral cortex development in mice. Microglia begin to enter the brain at embryonic day 9.5 (E9.5), and other immune cells, such as T cells, B cells and dendritic cells, infiltrate the brain at least by E16. Granulocytes and NK cells enter the brain later in development and are found in the adult brain. Immune cells, except microglia, are mostly located at the pial surface, ventricle and choroid plexus, and a few cells enter the brain parenchyma. The part of the figure below the timeline indicates the time course of major developmental events and the related immune. cells for each process. *E* embryonic, *P* postnatal, *MZ* marginal zone, *CP* cortical plate, *IZ* intermediate zone, *SVZ* subventricular zone, *VZ* ventricular zone. (From: Morimoto K and Nakajima K. 2019. Role of the immune system in the development of the central nervous system. Frontiers in Neuroscience. 13: 916 [219]]

the development of the immune system [220]. Glucocorticoids are anti-inflammatory and immunosuppressive, thus the perinatal activation of the HPA system can flood the brain with glucocorticoids and impair the development of both the innate and adaptive immune systems [220]. Likewise, estrogens and androgens both modulate the development of the innate and adaptive immune systems [221, 222]. During the period of sexual differentiation of the brain and body, the immune system is also sexually differentiated [223, 224] and the immune system may play a role in sexual differentiation (See Chap. 11).

The Neuroimmune System and NDD

Because of the close association between the nervous and immune systems during development and throughout the lifespan, the immune system plays a significant role in both neurodevelopmental and neurodegenerative diseases [225–228]. The function of the neuroimmune system is to protect the brain from disease, but the immune system is not fully functional until sometime after birth, leaving a window in which environmental toxins can attack the body without activating the immune system. This has both a positive and negative side. On the positive side, the adaptive immune system learns to discriminate between "good" and "bad" environmental chemicals and begins to develop antibodies to the bad molecules and tolerance towards the good [211, 229]. This so-called "neonatal window" is a critical period for immune system learning and memory which guides the activity of the innate and adaptive immune systems throughout the lifespan. During this time, the infant develops its own set of microflora which the immune system recognizes as "self" and tolerates. Failure of the immune system to learn to tolerate the normal microflora of the body leads to the development of autoimmune and allergic disorders [229]. The neural and endocrine systems interact with the developing immune system during this neonatal window.

On the negative side, exposure to toxins during the "neonatal window" of postnatal development, before the immune system has developed can cause infections which have long-term effects on neural development. Likewise, maternal infections during pregnancy can affect the embryo's neural development [228]. Prenatal infections which activate the immune system cause the release of a host of cytokines which affect all aspects of brain development, but have particularly devastating effects on those areas of neural development which are occurring during the infection [230, 231]. That is to say that infections which occur during particular critical periods of development (vulnerable periods) have their greatest effects on the neural features that are developing at that time and have a lifelong effect of neural function. Thus, perinatal immune activation may be a causal factor in a number of neurodevelopmental and neurodegenerative diseases [225]. Autism seems particularly sensitive to perinatal perturbations of the immune system [133, 232, 233].

Signaling Molecules as Epigenetic Transducers: Converting Environmental Signals to Gene Expression and Protein Synthesis

In order to activate the transcription of DNA to mRNA for protein synthesis, the exact section of DNA must be identified and the DNA "opened up" to allow the enzyme RNA polymerase to facilitate the replication of information on the DNA to mRNA. This requires epigenetic chromatin modification by histone molecules which regulate this transcription process [234, 235]. The intracellular signaling cascades activated by extracellular chemical signals binding to GPCR and the activation of transcription factors by steroid and thyroid hormones are, in a sense, epigenetic processes. Environmental stimuli (toxins, stressors, etc.) stimulate the release of extracellular chemical signals (NT, NP, hormones, etc.) which then activate the transcription factors and stimulate mRNA synthesis. In this way, environmental factors can regulate gene expression through epigenetic mechanisms. These mechanisms include histone methylation or acetylation, DNA methylation, etc. [235–238]. Epigenetic mechanisms mediate gene expression when NT bind to their receptors and are particularly potent in determining the course of synaptogenesis [239, 240].

Environmental stimuli which disrupt chemical signaling during pre- and/or post-natal development can lead to neurodevelopmental and neurodegenerative disorders [241–245]. These neuroendocrine disruptors act through epigenetic mechanisms to alter neurotransmitter, neuroendocrine and neuroimmune signals thus alter second messenger signaling, gene transcription and protein synthesis in the target cells. Disruption of these chemical signaling mechanisms during critical periods of development can result in neurodevelopmental and neurodegenerative disorders which last a lifetime [246, 247]. Thus, there is a neurochemical mechanism for the "developmental origins of health and disease" (DOHaD) hypothesis of NDDs [248–250]. Endocrine disruptors can affect a range of hormone targets, including adrenal, thyroid and gonadal steroid receptors, and act through epigenetic mechanisms (Fig. 1.6) to alter the course of brain development and sexual differentiation [251–256]. Epigenetic mechanisms underlying NDD are discussed in Chap. 8.

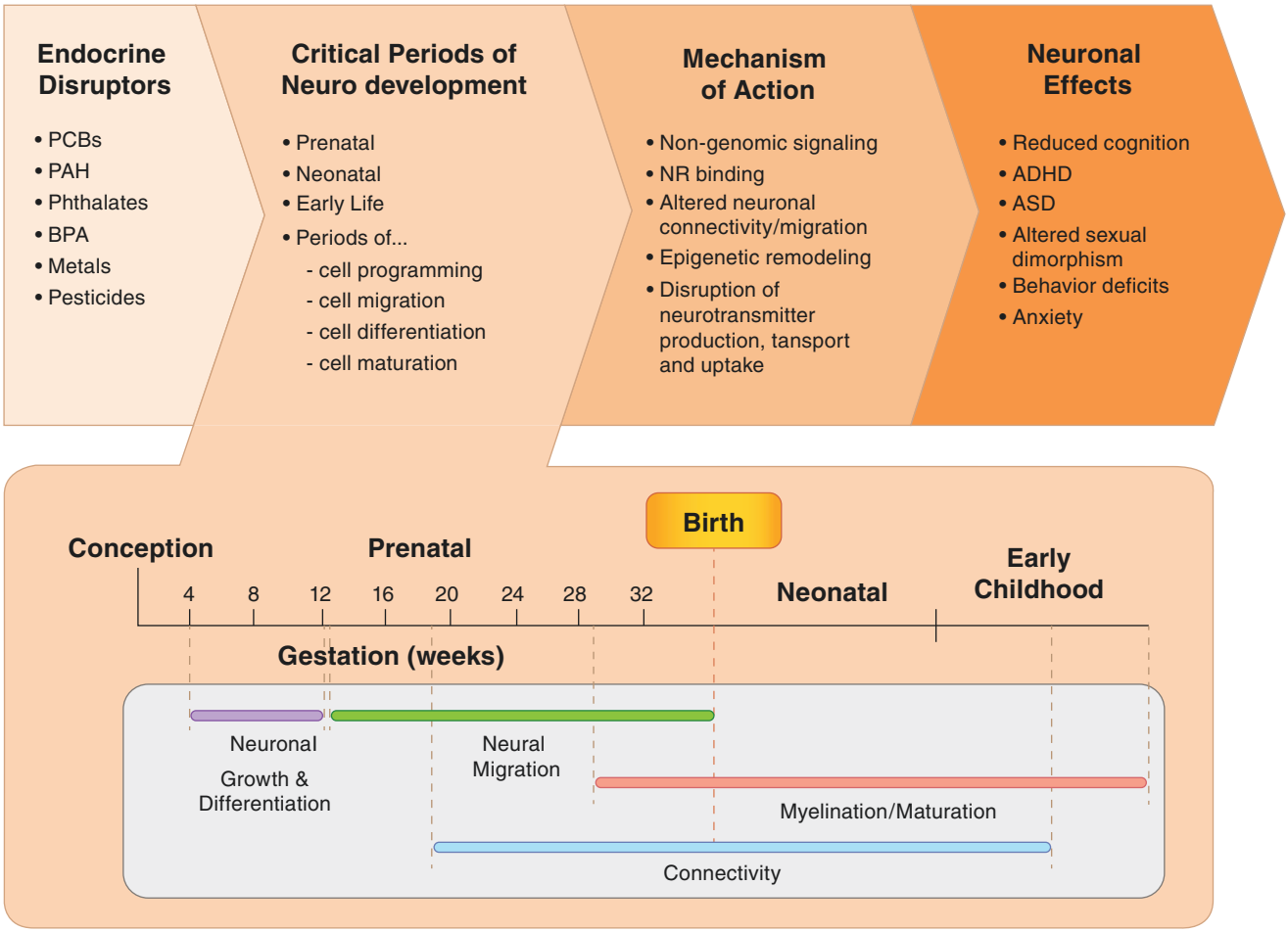

Fig. 1.6 An illustration of how exposure to endocrine disrupting chemicals during critical periods of development can lead to neurodevelopmental disorders. The mechanisms of action and neuronal effects of different endocrine disruptors during the pre-natal and perinatal critical periods of development are shown along the time line from conception to early childhood in humans. (From: Schug TT, Blawas AM, Gray K, Heindel JJ, Lawler CP. 2015. Elucidating the links between endocrine disruptors and neurodevelopment. Endocrinology. 156: 1941–1951 [76]]

Critical (Sensitive) Periods in Development

The temporal and spatial development of the brain occurs through a number of stages which have been called critical or sensitive periods (Figs. 1.1 and 1.7). During these periods, the brain is particularly responsive to internal and external stimuli (or the lack of such stimuli) which modify neural growth and behavioral development [257–259]. A *critical period* is the time during which the presence of specific external or internal stimuli is necessary for the normal development of the nervous system and a *sensitive period* is the longer period during which these stimuli can modulate the developing nervous system by causing alterations or reorganization of the nervous system [260–262]. Vulnerable and optimal periods are special cases of critical periods. The *vulnerable period* is a time when the developing nervous system can be adversely affected by the lack of required stimuli or by teratogens [263]. An *optimal period* is the time when the developing nervous system will be positively affected by an external stimulus. A majority of the critical periods occur during embryonic and fetal development, and the sensitive periods for the development of the CNS, ears and genitals can extend into postnatal development. Because each aspect of physical, neural and behavioral development shows a different pattern of growth after conception, there are many different "critical periods" during development. There are also postnatal critical periods for the development of sensory, motor, cognitive and social behaviour (Fig. 1.7) which depend on the timing of the development of the appropriate neural circuits via mechanisms of synaptic plasticity and external stimulation from the environment [264–266].

The timing of critical periods depends on the action of chemical signals at their receptors on target cells and the activation of intracellular messengers to initiate gene tran-

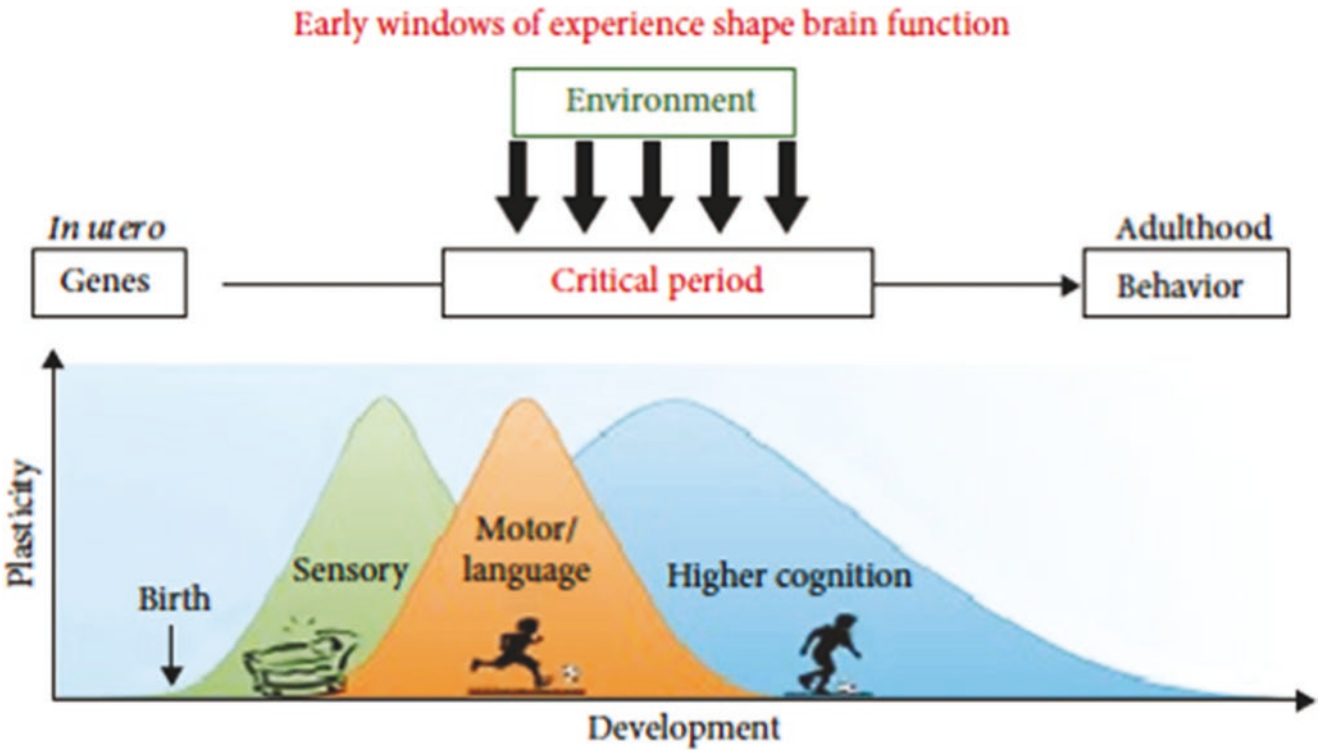

Fig. 1.7 An illustration of the interaction between gene expression and experience with environmental stimuli during critical periods in postnatal sensory, motor and cognitive development. (From: Nelson CA 3rd, Zeanah CH, Fox NA. 2019. How early experience shapes human development: the case of psychosocial deprivation. Neural Plast. 2019: 1676285 [301]]

scription and protein synthesis [19, 267–270]. One of the most important neural events during critical periods is synaptogenesis and the development of different patterns of excitatory and inhibitory (Gaba/glutamate) synapses [271]. The onset and offset of critical periods may be determined by the timing of the development of excitatory and inhibitory synapses: the E/I ratio, in which GABA plays an important role [99, 272–274]. Based on the hypothesis that synaptic organization defines the timing of the onset and offset of critical periods in neural development, internal stimuli (such as chemical signals) and external stimuli (such as nutrients, bacteria, viruses, neurotoxins or endocrine disrupting chemicals), which affect the balance of E/I synaptic organization during these critical periods, can facilitate or impair neural development [275].

During critical periods neurotrophic factors, neurotransmitters, hormones and gliotransmitters interact in the regulation of brain development as shown in Fig. 1.1 [18, 276]. Critical periods are a time of extreme synaptic plasticity, during which neural circuits are modified under the guidance of astrocytes at the tripartite synapses. There are also critical periods in development for neural-endocrine and neural–immune interactions, in which thyroid hormones [45, 182, 267], adrenal hormones [186, 187], gonadal hormones [277–279] and cytokines [280–282] interact to regulate neural development. Because of the importance of the timing of critical and sensitive periods, a great deal of effort has been made to understand the parameters defining them [257, 272, 283–285].

Sex differences in the neural circuits of the brain develop through the influence of hormones and cytokines on neural development during three critical (sensitive) periods: (1) a prenatal critical period during which androgens masculinize males; (2) a postnatal sensitive during which estrogens feminize females; and (3) a pubertal sensitive period during which secondary sex characteristics develop and sex differences in the brain and neuroendocrine system become activated as discussed in Chapter 11 [286, 287].

Puberty as a Critical Period in Brain Development

Since the brain develops throughout childhood and enters a new critical period of organization at puberty, we should think of puberty as much more than simply the period of sexual development; it is a period of significant brain reorganization. In addition to the activation of secondary sexual characteristics, puberty can also result in the onset of adolescent neurodevelopmental disorders such as schizophrenia [288–292]. Because there is significant synaptic remodeling at puberty, there is the "window of vulnerability" for the

development of synaptopathies and for disruption of excitatory: inhibitory signaling ratios. Finally, because the neuroendocrine changes that occur at puberty are all regulated by chemical signaling pathways, there is the opportunity for environmental epigenetic mechanisms to disrupt the neuroendocrine system during this critical period of brain development [284, 293].

Puberty is a critical period for brain reorganization and development from the brain of childhood to the brain of adulthood; a period when the neural circuits underlying higher order cortical functions develop [216, 292]. Structural and functional changes occur in neural circuits in the prefrontal cortex, amygdala and hippocampus during the adolescent critical period that begins with puberty [294] and the activational effects of the sex hormones on these circuits as they are reorganized results in the sexual differentiation of the adult brain [295, 296]. The neural reorganization that occurs at puberty involves the neural, endocrine and immune systems. Changes in a wide range of chemical signals, including Dopamine, GABA, glutamate, and BNDF (Fig. 1.8a) underlie the reorganization of the neural circuits and synaptic changes that occurs during the adolescent critical period of brain development [295, 297]. [216, 296]). These neural, endocrine and immune changes result in changes in cognitive, social and emotional behaviour, which is reflected in changes in EEG patterns during sleep [289, 298].

The negative side of the critical periods of neural reorganization in early postnatal development and at puberty is that the brain is vulnerable to abnormalities in synapse and neural circuit development due to environmental stimuli [17, 263]. The developing brain is susceptible abnormal development due to the influence of neurotoxins [299], neuroendocrine disruptors [76], malnutrition [300]; as well as environmental and social stressors (Nelson 3rd and Gabard-Durnam [301]).

Critical Periods and Neurodevelopmental Disorders

Understanding the neurobiological mechanisms underlying critical periods and how they are disrupted may provide a window to understanding neurodevelopmental disorders [17, 251, 284]. Because the development of neural circuits underlying brain functions depends on the development of synapses that connect neurons within a neural network, the critical periods for brain development may depend on synaptogenesis, synaptic pruning and synapse elimination [99]. Environmental stimuli which modulate synapse development during these critical periods may have long-term effects on neural circuit functions. For example, environmental enrichment facilitates synaptic plasticity and may lead to long-term enhancement of neural development [302]. On the other hand, teratogenic chemicals and other toxic stimuli (including bacteria, viruses, and physical or mental stress) may interfere with synaptogenesis (but has been expanded to cover all aspects of neural development over different time periods [303, 304] and disrupt the E:I ratio of synapse development, resulting in neural networks that underlie neurodevelopmental (and neurodegenerative) disorders. Indeed, one could argue that synaptic disruption is the fundamental neural change in responses to environmental input as altered synaptic function will disrupt entire neural networks [305]. It is clear that there are critical periods for the development of many neural and non-neural systems in the body and that these occur on different time scales [303, 304].

Autism and Schizophrenia as a critical period disorders. As mentioned above, puberty marks the beginning of the adolescent critical period of brain reorganization and thus opens a "vulnerable period" for the development of abnormal neural networks which could underlie adolescent-onset neuro-psychiatric and addictive disorders [306–308]. This neural reorganization at puberty involves glial cells as well as neurons: astrocytes are involved in shaping the tripartite synapses, microglia regulate synapse elimination and oligodendrocytes myelinate axons [140, 217, 309].

It is no surprise to find that structural connectivity within the prefrontal cortex and the frontoparietal neural network continues to change throughout adolescence and early adulthood in people with autism spectrum disorder [310]. Although it is most likely that autism is the result of abnormal cortical circuit development during perinatal critical periods [311, 312], the neural circuits underlying social and repetitive behaviors characteristic of autism may be further re-organized during the adolescent critical period, a time when sex differences may emerge [310, 313, 314]. This suggests that, in addition to the perinatal critical period of brain development [315, 316], the "critical period" of brain reorganization at puberty may have a significant influence on the development of schizophrenia [217, 317–319]. Since glial cells are intricately involved in neural development at both perinatal and adolescent critical periods (Fig. 1.8a–c), they may play a significant role in the development of NDD such as schizophrenia [309].

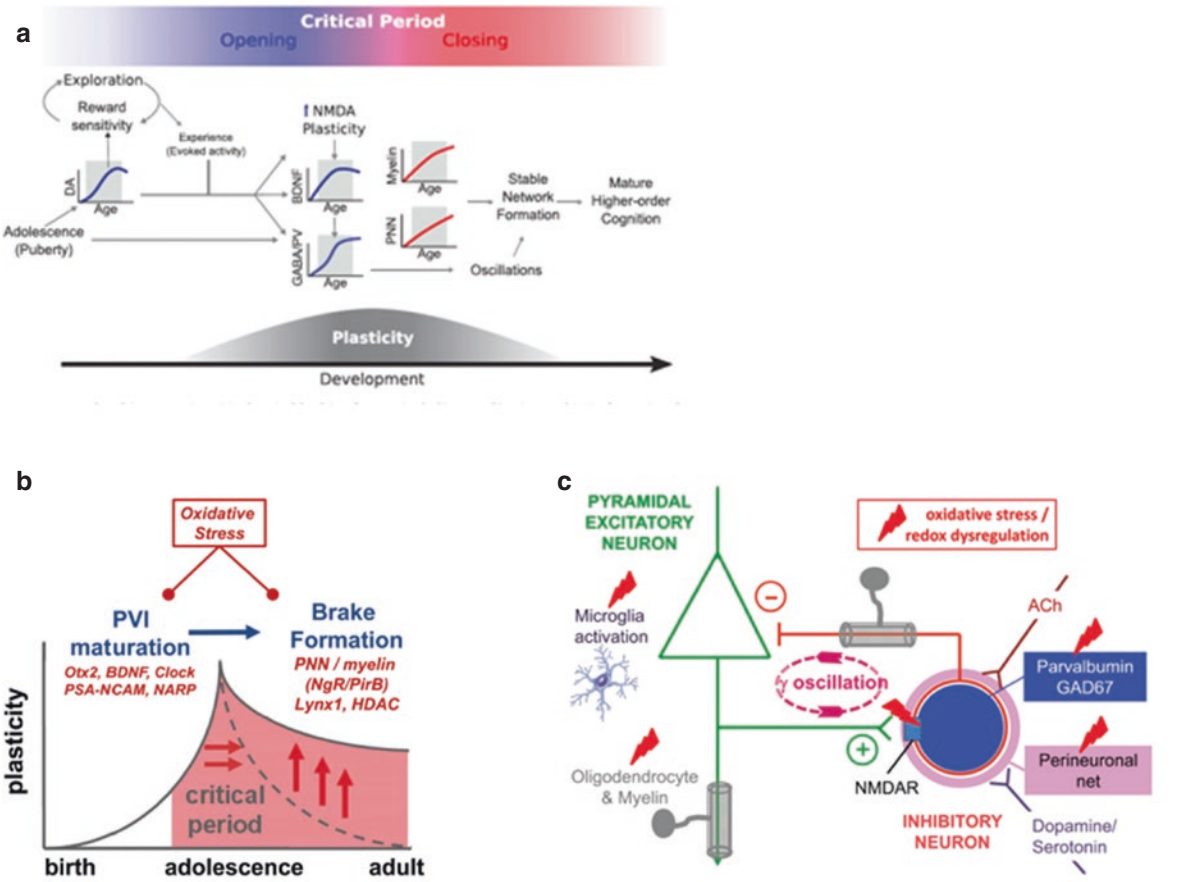

Fig. 1.8 The reorganization of neural circuits during critical periods of development at puberty. (**a**) Adolescence begins with the onset of puberty and a concomitant increase in dopamine (DA) availability. Increases in DA motivate exploratory behavior and heightened reward reactivity which, in turn, promote the experience accumulation necessary to shape experience-dependent plasticity. Increased DA levels, hormonal changes at puberty, and novel experiences may interact with neurobiological factors that facilitate critical period (CP) plasticity. These facilitating factors include changes in NMDA signaling and receptor concentrations that promote experience-dependent plasticity, increased levels of brain-derived neurotrophic factor (BDNF), and maturation of GABAergic inhibitory circuitry (particularly parvalbumin positive (PV) interneurons). The maturation of inhibitory circuitry has important functional consequences including a reduction in the excitation-to-inhibition balance (E/I balance) and facilitation of high-frequency oscillatory capability of local circuits. As the critical period progresses, age-related increases in critical period braking factors, including myelination and perineuronal net (PNN) formation, begin to restrict further plasticity to close the CP window and stabilize circuits into adulthood. This stabilization leads to consistent and reliable circuit function and communication which underlies the stabilization of cognitive ability that is characteristic of mature higher-order cognitive function. Blue and red curves represent the development of facilitating and braking factors, respectively. The shaded grey area reflects the adolescent period. (From: Larsen B, Luna B. 2018. Adolescence as a neurobiological critical period for the development of higher-order cognition**.** Neurosci Biobehav Rev. 94: 179–195. [216]]. (**b**). Proposed mechanism for oxidative stress to regulate adolescent critical period plasticity underlying the endophenotype for schizophrenia. Schizophrenia symptoms may reflect delayed plasticity due to a failure of critical period onset and/or closure. Disease etiologies may dysregulate the expression of molecular brakes which normally follow parvalbumin-positive interneuron (**PVI**) maturation and extend developmental plasticity. Ultimately, this would destabilize circuit function in the face of undesirable information, as seen in mental illness. A common mechanism impacting PVI/perineuronal nets (PNN)/myelin is redox dysregulation, which represents a novel target for preventive neurodevelopmental intervention. Alternatively, once PVI functional impairment is detected (e.g., mismatch negativity [MMN], γ-oscillations), a supplemental reinforcement of molecular brakes on plasticity may be considered. [From: Do KQ, Cuenod M, Hensch TK. 2015. Targeting oxidative stress and aberrant critical period plasticity in the developmental trajectory to schizophrenia. Schizophr Bull. 41: 835–46 [217]]. (**c**). A schematic representation of the impact of oxidative stress/redox dysregulation on cortical microcircuits, including excitatory pyramidal and inhibitory parvalbumin-positive interneuron (PVI) connected reciprocally and supporting γ-oscillations. Oxidative stress/redox dysregulation activates inflammatory microglial cells, and with the N-methyl-d-aspartate receptors (NMDAR), reducing their activity, leading to a damaging potentiating effect. As a consequence, PVI surrounded by their perineuronal nets and myelin-forming oligodendrocytes are impaired, as manifested by alterations of local oscillations and distant synchronization. These cellular and molecular changes are known to alter the timing of critical periods. Microcircuits are also affected by cholinergic (Ach), dopaminergic and serotonergic inputs. + = excitatory input; − = inhibitory input. BDNF = Brain-derived neurotrophic factor; Clock PSA-NCAM = clock gene encoded polysialylated-neural cell adhesion molecule; GAD67= glutamic acid decarboxylase molecular weight 67; HDAC = histone deacetylase; Lynx1= a gene encoding the Ly6/neurotoxin 1 protein; Myelin (NgR/PIRB)= the myelin-associated Nogo receptor and paired immunoglobulin-like receptor; NARP = Neuropathology, ataxia and retinitis pigmentosa. Otx2 = Orthodenticle Homeobox 2 Protein Coding gene. [From: Do KQ, Cuenod M, Hensch TK. 2015. Targeting oxidative stress and aberrant critical period plasticity in the developmental trajectory to schizophrenia. Schizophr Bull. 41: 835–46 [217]]

Summary

The purpose of this chapter was to give an overview of brain development which is fundamental to understanding the neurobiological basis of neurodevelopmental disorders. While defining the brain may seem simple, there are many ways to understand the mechanisms regulating neural development and its disorders. Likewise there are many types of neurons, chemical signaling molecules, synapses and neural circuits in the brain and disruption in any of these may lead to NDD. Often neglected, the glial cells, endocrine, and immune systems are also involved in the regulation of neural development and disruption of these systems can also lead to NDD. Neural development occurs during critical or sensitive periods, and during these periods the brain is sensitive to environmental stimulation which can facilitate (enrichment) or impair (neurotoxins) neural development. One finding that stands out in the study of neural development is the importance of synapse formation and modulation for the creation of functional neural circuits that underlie cognitive functions. The following chapters in this book examine the development of specific neural circuits underlying NDD.

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Multiple Choice Questions (* = correct answer)

1. Which of the following is NOT a phase of brain development?
(a) Neurogenesis
(b) Gliogenesis
(c) Neural migration
(d) **Neurotoxicosis**
(e) Neural differentiation

2. Which of the following is a chemical signal in the brain?
(a) GABA
(b) BDNF
(c) Substance P
(d) Testosterone
(e) **All of the above**

3. What are the three parts of a tripartite synapse? (pick 3)
(a) **Pre-synaptic neurons**
(b) **Post-synaptic neurons**
(c) **Astrocytes**
(d) Oligodendrocytes
(e) The amygdala

4. Which of the following types of glial cell gives rise to neurons?
(a) Oligodendrocytes
(b) Microglia
(c) Astrocyte
(d) **Radial glial cell**
(e) None of the above

5. Which of the following best describes a time during development when the brain is most responsive to environmental neurotoxins?
(a) Critical period
(b) **Vulnerable period**
(c) Sensitive period
(d) Optimal period
(e) Puberty

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Neural Induction and Regionalization

2

Jeffrey T. Wigle and David D. Eisenstat

Learning Objectives

- Learn the early stages of nervous system development, especially neural induction and early regionalization of the nervous system to the forebrain, midbrain, hindbrain, and spinal cord, from which all differentiated and functional nervous system tissues are derived.
- Identify some of the key molecules and signaling pathways essential for nervous system development, including the sonic hedgehog (SHH), transforming growth factor beta (TGFβ), canonical WNT, and Notch/Delta signaling pathways.
- Appreciate the advantages and limitations of using various invertebrate (Drosophila, *C. elegans*) and vertebrate (chick, *Xenopus laevis*, zebrafish, and mouse) model systems to understand early nervous system development in the human.
- Link some clinical disorders of early nervous system development to specific molecules and signaling pathways described in this chapter.

Highlights

- **Table** comparing two invertebrate (fruit fly and nematode) and four vertebrate (chick, frog, zebrafish, and mouse) model systems.
- Description of the genes and signaling pathways disrupted in some **disorders linked to early nervous system development**, including neural tube defects, holoprosencephaly, hydrocephalus, and neuronal migration disorders.
- Description of recent work using **induced pluripotent stem cells** (iPSCs) and **brain organoids** to improve our understanding of early nervous system development.

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Introduction to the Neural Tube and Early Regionalization of the Central Nervous System

The vertebrate central nervous system (CNS), incorporating the brain and spinal cord, begins as an epithelial sheet and through overlapping stages of neural induction, regionalization, and patterning, dorsal/ventral and anterior/posterior axes are established. Within each prospective CNS region, the prosencephalon (forebrain), mesencephalon (midbrain), metencephalon (cerebellum), rhombencephalon (hindbrain) and myelencephalon (spinal cord), neural progenitor cells (NPC)

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are generated, proliferate, undergo apoptosis, and migrate. These progenitors differentiate into neuronal and glial cell populations as well as extend axons, commence myelination, and establish synaptic connections. The prosencephalon will later be further regionalized into the telencephalon (including the neocortex and germinal matrices) and diencephalon (including the thalamus and hypothalamus). **Primary neurulation** involves fusion of the neural tube in the dorsal midline at three sites of closure in the following temporal sequence: (1) hindbrain/cervical boundary, (2) forebrain/midbrain boundary, and (3) rostral end of the neural tube [1].

The topics of CNS stem cells (Chap. 3), neurotrophins and cell death (Chap. 4), synaptogenesis (Chap. 5), axonal guidance (Chap. 6), and myelination (Chap. 7) are covered separately in subsequent chapters. This chapter will provide an overview of current concepts regarding induction, early regionalization, and patterning of the central nervous system, including discussion of the key morphogens and signaling pathways involved in these processes. In addition, congenital malformations and related disorders resulting from dysregulated neural induction, early regionalization, and patterning will be briefly reviewed.

Neural Induction

Model Systems: *Drosophila*, *C. Elegans*, *Xenopus*, Chick, and Mouse

Most of what we understand about neural induction has been learned from the use of invertebrate (*Drosophila melanogaster*, *C. elegans*) and vertebrate (*Xenopus laevis*, chick and mouse) model systems. Key facts about each model system, including advantages and disadvantages for their use in research, are presented in Table 2.1.

Early embryologic researchers proposed the "default model" of neural induction, wherein in the *absence* of specified signals favoring **bone morphogenetic protein (BMP)** signaling, the ectoderm gives rise to the neural plate [2, 3]. However, depending on the model system and experimental design used, the results obtained cannot always be explained by a simple default model of neural induction [2].

Setting Up Anterior/Posterior and Dorsal/Ventral Axes

How the differentiated CNS is generated from an unspecified sheet of epithelial cells has fascinated human embryologists, developmental biologists, and neuroscientists for decades. The developmental anatomy and ease of experimental manipulation of *Xenopus* and chick model systems permitted earlier investigators to elegantly spatiotemporally identify critical regions from which neural inducers originate by transplanting donor tissues from relevant developmental timepoints and anatomical areas.

By convention, *dorsal* is defined by the side in which the sperm fertilizes the *Xenopus* egg with *ventral* being directly opposite. Initially, the unfertilized *Xenopus* embryo has animal (anterior) and vegetal (posterior) poles, from which ectoderm and endoderm will be derived during gastrulation, respectively. From the ectoderm are derived the epidermis giving rise to skin and dermal tissues and the nervous system. Induction of the mesoderm, which gives rise to the notochord (most dorsal region), somites, and mesenchyme (eventually the skeleton, muscle, kidney, heart and blood in the mature animal), follows from the involuting marginal zone (IMZ) between the ectoderm and endoderm first specified during the blastula stage. Subsequently, signals from the dorsal lip of the *blastopore* are instructive for specifying the presumptive neurogenic region as gastrulation proceeds. In classic experiments, isolated late blastula stage *Xenopus* animal caps become epidermis, whereas gastrula-derived animal caps become neural tissue [4].

Nodes and Organizers

In *Xenopus*, the **Spemann organizer** from the dorsal lip of the blastopore *dorsalizes* adjacent mesoderm by inhibiting ventral signals from the mesoderm. The inductive properties of the organizer change during gastrulation. In the famous Spemann and Mangold experiment, when taken from the early gastrula, a graft from this organizer region translocated to the ventral side induces a second anterior/posterior (A/P) axis including a second neural tube. However, when derived from the late gastrula stage, a similar graft only induces the formation of tail structures.

Identified molecules within the Spemann organizer include those secreted from the notochord, such as chordin, noggin, and follistatin. Both chordin and noggin specifically block BMP family members, including BMP-2, BMP-4, and BMP-7. BMPs are members of the **transforming growth factor beta (TGFβ) superfamily** that are anti-neuralizing. Follistatin, also known as activin-binding protein, binds to activin, similarly interfering with TGFβ signaling. Acting downstream of BMP and TGFβ receptor signaling are the **SMADs**, vertebrate homologs of *mad* (*mothers against decapentaplegic*), the *Drosophila* homolog of TGFβ. Of the nine members of the SMAD family of transcription factors are the R-SMADS (receptor-regulated; *Smads*-1, 2, 3, 5, 8, 9), the I-SMADS (inhibitory; *Smads*-6, 7), and one co-SMAD (common partner; *Smad*4) [5](Fig. 2.1).

In the chick (*Gallus gallus*) and mouse, neural induction proceeds differently when compared to the process in *Xenopus*. **Hensen's node** arises from the most anterior end

Table 2.1 Comparison of model organisms

Organism	Fruit fly	Nematode	Mouse	Chick	Frog	Zebrafish
Latin name	*Drosophila melanogaster*	*Caenorhabditis elegans*	*Mus musculus*	*Gallus gallus*	*Xenopus laevis*	*Danio rerio*
Life cycle	Gastrula—3 h after fertilization Hatching—16-20 h 3 larval stages and pupation Metamorphosis Adult >9 days	Gastrula—5-10 h after fertilization Hatching −15-20 h 4 larval stages Adult - > 3 days	Gastrula—7 days after fertilization Birth—19 days Adult—6–8 weeks	Gastrula—16 h after fertilization Hatching—6 days after laying Adult—60 days after laying	Gastrula—15 h after fertilization Tadpole—4 days Metamorphosis Adult—60 days	Gastrula—8 h after fertilization Free swimming 2 days after fertilization Adult—90 days
Triploblast	Yes	Yes	Yes	Yes	Yes	Yes
Genome	15,431 genes	21,187 genes	33.4 K – 36.5 K genes	17,529 genes	>20 K (X. Tropicalis)	28,770 genes
Chromosomes	4 pr; polytene	6 pr	20 pr	39 pr	36 pr	25 pr
Advantages as a model system	– short life cycle – inexpensive – can be grown in large numbers – forward and reverse genetics – large scale mutagenesis – microsurgical manipulation is possible	– short life cycle – inexpensive – can be grown in large numbers – forward and reverse genetics – transparent embryos – large scale mutagenesis – invariant lineage – targeted ablation of individual cells – simple anatomy	– can model human disease – reverse genetics (gain/loss of function; conditional knockouts/ knockins) – many antibodies are available	– similar to mammals in complexity – less expensive to maintain than mice (incubator) – large eggs permit invasive procedures (transplantation, retrovirus injection, electroporation of nucleic acids) – later development is similar to mammals	– can develop in tap water – inexpensive – large, fertilized eggs easy to obtain – embryos are – hardy/ resist infection – can manipulate oocytes (mRNA injection) – fragments of early embryos can be cultured in simple media	– embryonic development is external – embryos are large and transparent – relatively inexpensive to maintain – arge number of offspring – forward genetics (mutagenesis screens)
Disadvantages as a model system	– complex anatomy – difficult to model human disease – limited antibodies	– difficult to model human disease – organs not similar to mammals – few antibodies	– expensive to maintain – small litters – forward genetics – susceptible to infections – embryonic development is internal – intrauterine manipulation is difficult – large mutagenesis screens are difficult	– forward/reverse genetics not routine – transgenic approaches are not currently an option – very early development occurs in the oviduct; difficult to study	– forward/reverse genetics not routine – limited antibodies for protein expression – later development is less similar to mammals – transgenic approaches are only a recent option and are not widely available	– reverse genetics (but can use morpholinos for knock-downs) – few antibodies – small size – may not be as useful as mouse to model human disease

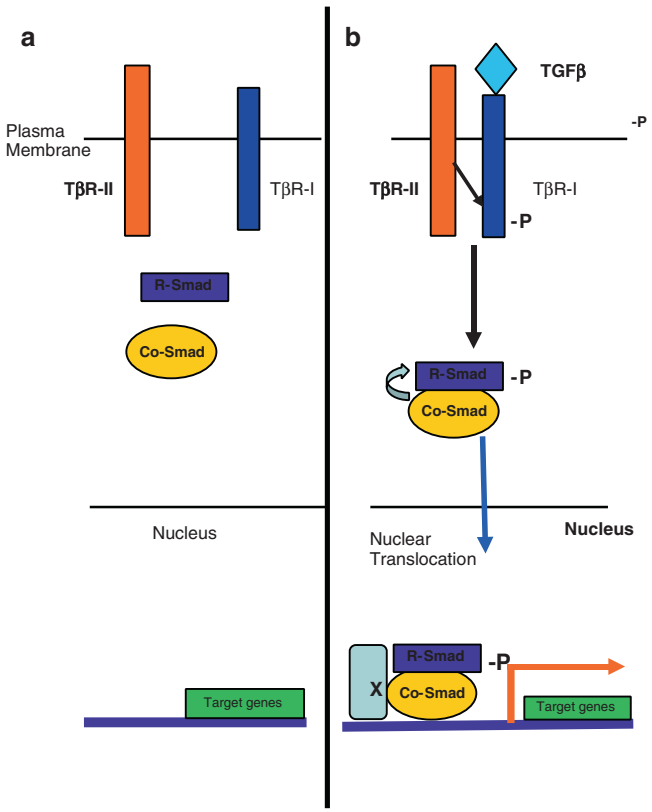

Fig. 2.1 The transforming growth factor (TGFβ) signaling pathway. (**a**) TGF-β receptor subunit type II (TβR-II) is constitutively active. (**b**) Type I TGF-β receptor subunits (TβR-I) are recruited to form a heterodimeric receptor complex upon binding of ligand to TβR-II, with transphosphorylation (-P) of the TβR-I kinase domain. R-Smads are subsequently phosphorylated by signaling from the activated receptor complex; R-Smads then bind to a co-Smad, translocate from the cytoplasm to the nucleus, and activate gene transcription with cofactor(s). [With Permission from Wigle JT and Eisenstat DD. In Moore, Persaud, and Torchia, Editors, The Developing Human, 11th Edition. Fig. 21.4, Page 466. Copyright Elsevier: Saunders [5]]

of the primitive streak (PS). The PS begins to regress after extending halfway across the blastoderm. Hensen's node subsequently moves posteriorly as the head fold and **neural plate** begin to form. As this node moves backward, the **notochord** develops anterior to it and somites begin to form on either side of the notochord. Once the notochord has formed, neurulation begins, following the progress of the notochord in an anterior to posterior direction. Posterior to Hensen's node, notochord formation, somite formation, and neurulation have not yet begun. Hensen's node can induce a new A/P axis in avian embryos. Transplants of tissue containing Hensen's node obtained from a donor quail embryo induce a second A/P axis in a chick host at the primitive streak stage. In a latter variant of the Spemann–Mangold experiment, Hensen's node explants from a chick epiblast sandwiched between *Xenopus* late blastula animal caps induce neural gene expression; however, explants derived from the posterior primitive streak or non-primitive streak epiblast cannot induce neural genes [4–7].

Inducers, Morphogens, Gradients, and Signaling Pathways

Developmental biologists have defined three criteria for an *inducer*. (1) The molecule has the correct spatial, temporal, and quantitative expression. Experimentally, this can be determined by *in situ* RNA hybridization, immunohistochemistry using specific antibodies, or more recently, by single-cell RNA sequencing. (2) Appropriate cells can respond to the factor. For example, using *Xenopus*, one can apply the candidate factor to isolated animal caps in culture or inject mRNA encoding the candidate factor into animal pole cells of the early blastula. (3) Blocking the function of the inducer factor prevents induction from taking place. This blockade can be accomplished by use of antisense oligonucleotides, RNA interference, CRISPR-*Cas9*-mediated gene editing, blocking antibodies, or dominant negative (e.g., mutant) receptors [4].

Important molecules isolated from Spemann's organizer, Hensen's node and/or the notochord include *Brachyury (a T-box gene)*, *Goosecoid* (a homeobox gene), *Hnf-3β* (an Hnf-class homeobox gene), and *Lim-1/Lhx1* (a Lim-class homeobox gene) and secreted proteins Nodal and Sonic Hedgehog (Shh).

Gradients of Nodal, a member of the TGFβ superfamily that binds to activin-type receptors, in the mesoderm (ventral, low to dorsal, high) may be specified by canonical **Wnt pathway** signaling mediated via nuclear translocation of β-catenin [5](Fig. 2.2).

Interestingly, *noggin* mRNA injected into early gastrula *Xenopus* embryos ventralized by ultraviolet (UV) treatment rescued neural induction in a manner similar to injections of polyA mRNA derived from the mesoderm of hyperdorsalized embryos resulting from treatment with lithium. Lithium inhibits glycogen synthase kinase-3 beta (GSK-3β), integral to both canonical Wnt and other signaling pathways, such as Shh. As stated earlier, intact animal caps cultured *in vitro* become epidermis, whereas dissociated cells from animal caps become neural tissue. However, adding BMP-4 to these dissociated cells blocks neural induction. In support of these experiments, expression of mRNA encoding a truncated activin receptor induces neural tissue when injected in isolated animal caps taken from *Xenopus* oocytes [2, 8].

Retinoids

Retinoids, including vitamin A (retinol) and 13-*cis*-retinoic acid, play an important role in establishing the A/P axis of the central nervous system and can serve as teratogens during early pregnancy. Retinoic acid "posteriorizes" the A/P axis, and either excessive retinoic acid or inhibition of its degradation leads to posteriorized structures. However, low levels of retinoic acid or defective endogenous retinoic acid synthesis will lead to a more "anteriorized" AP axis. Retinoic acid binds to its intracellular receptors, thereby regulating

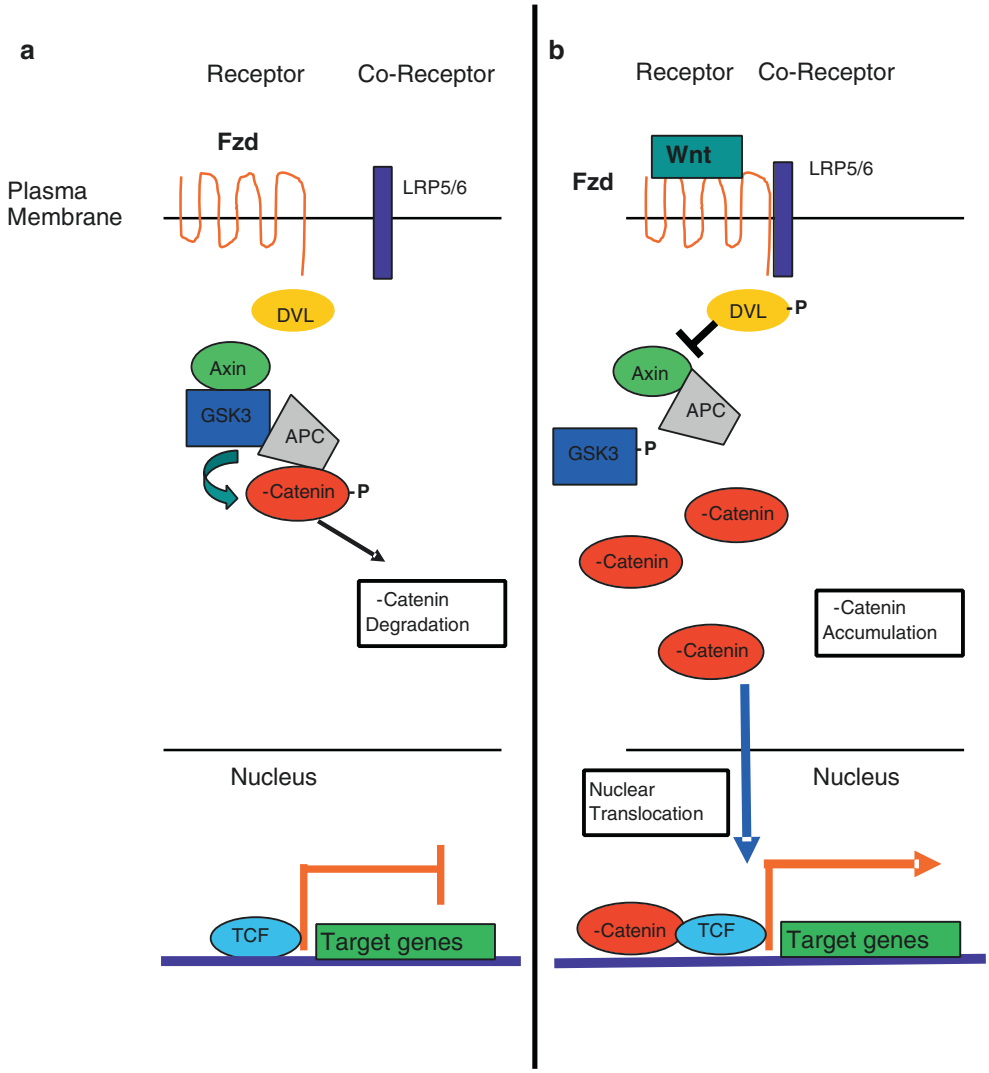

Fig. 2.2 The classic (canonical) Wnt signaling pathway. (**a**) When the Wnt ligand is not bound to the Frizzled (Fzd) receptor, β-catenin is phosphorylated (-P) by a multiprotein complex and targeted for degradation. Target gene expression is repressed by T-cell factor (TCF). (**b**) When Wnt is bound to Fzd, there is recruitment of LRP co-receptors, subsequent phosphorylation of Disheveled (DVL), and accumulation of β-catenin in the cytoplasm. β-catenin can translocate into the nucleus to activate expression of its target genes. *APC,* Adenomatous polyposis coli; *GSK-3,* glycogen synthase kinase 3; *LRP,* lipoprotein receptor–related protein. [With Permission from Wigle JT and Eisenstat DD. In Moore, Persaud, and Torchia, Editors, The Developing Human, 11th Edition. Fig. 21.6, Page 469. Copyright Elsevier: Saunders [5]]

the expression of downstream genes, including members of the *Hox* gene family of transcription factors [5].

Vertical *Versus* Planar Neural Induction

There are several postulated mechanisms of neural induction of anterior ectoderm from the underlying mesoderm and subsequent patterning of the early **neural tube**. These mechanisms may be dependent upon the experimental model systems used. In classical vertical or transverse neural induction, there is direct patterning of the overlying ectoderm by graded dorsoventral signals within the mesoderm. This patterned neuroectoderm subsequently regionalizes the neural tube along the A/P axis. In non-classical planar neural induction, these neural induction signals are derived from within the neural plate itself. These experiments were initially performed by sandwiching two explants from the dorsal blastopore lip containing IMZ cells of the early *Xenopus* gastrula (i.e., Keller Sandwiches) [4, 8].

Lateral Inhibition and Notch Signaling

Sox genes, members of the SRY high mobility group (HMG) family of transcription factors, are sufficient to induce neural differentiation through upstream activation of proneural genes such as neurogenin. In cells with activated BMP or Wnt signaling pathways, downstream expression of transcription factors such as GATA and MSX represses expression of *Sox* genes and these cells become epidermis. However, if **fibroblast growth factor (FGF)** signaling through FGF receptors is active or BMP signaling is blocked by inhibitor molecules such as noggin, chordin, or follistatin expressed from the organizer region, then *Sox* genes and subsequently downstream proneural genes are expressed [4, 8].

Furthermore, neural progenitor specification within the presumptive neuroepithelium occurs through **lateral inhibition**, a complex feedback loop process which is remarkably conserved from invertebrates to vertebrates. Conceptually,

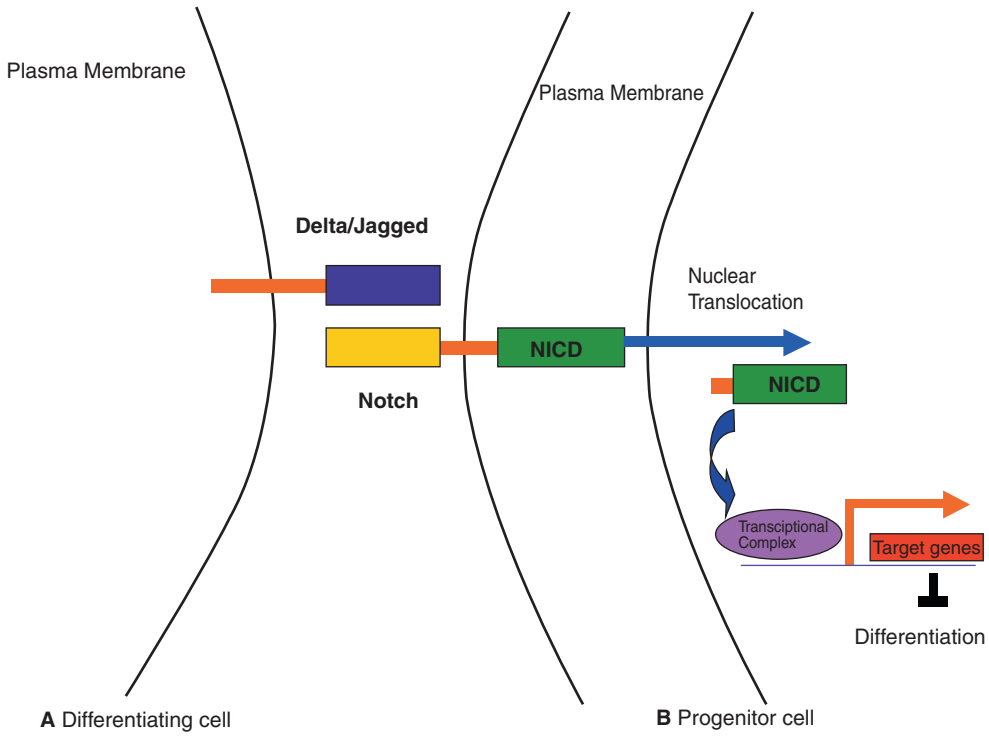

Fig. 2.3 The Notch/Delta signaling pathway. Left. Notch signaling is not active in differentiating cells. Right. In progenitor cells, Notch signaling results in cleavage of the Notch intracellular domain (NICD). Subsequently, there is translocation of the NICD to the nucleus, binding to a transcriptional complex resulting in expression of target genes, such as the bHLH gene *Hes1*, that inhibit differentiation. [With Permission from Wigle JT and Eisenstat DD. In Moore, Persaud, and Torchia, Editors, The Developing Human, 11th Edition. Fig. 21.9, Page 471. Copyright Elsevier: Saunders [5]]

one of the best described examples is in *Drosophila* sensory organ precursor specification, wherein one neuroblast is specified by cell–cell interactions within a proneural cluster and subsequently delaminates; the remainder of the cells within the cluster becomes epidermal cells, considered as a "default" cell fate. Some important *proneural* genes, such as those from the *achaete-scute* complex, are encoded by members of the basic helix–loop–helix (bHLH) family of transcription factors; these bHLH molecules dimerize and bind directly to DNA to regulate transcription of their target genes. Proneural mutants do not generate neuroblasts, only epidermal cells. Furthermore, mutations of *neurogenic* genes encoding members of the **Notch-Delta signaling pathway** result in the generation of excessive neuroblasts within a proneural cluster [4] (Fig. 2.3).

In the differentiating cell "A" destined to become a neuroblast, expression of Achaete-Scute proteins activates the Delta ligand expressed on its cell surface. Delta subsequently binds to its cognate Notch receptor expressed on the surface of the adjacent cell "B"; downstream signaling via cleavage of the Notch intracellular domain (NICD) leads to inhibition of proneural gene expression within cell "B," thereby leading to reduced activity of Delta–Notch signaling in cell "A" that will become a neuroblast. In vertebrates, the key bHLH transcription factor regulated by Delta–Notch signaling is neurogenin, which is upstream of NeuroD.

Asymmetric Versus Symmetric Cell Divisions

Another mechanism that is highly conserved from invertebrates to vertebrates is asymmetric cell division to specify a differentiated neuron from a neuroblast. There is a well-described phenomenon known as **interkinetic nuclear migration** in the developing neuroepithelium wherein early apical/basal cell polarity is established by the apical/basal migration of the nucleus within the cell during various phases of the cell cycle. M-phase (mitosis) occurs at the apical aspect directly adjacent to the ventricular surface, whereas S-phase occurs at the basal aspect. Furthermore, in the ventricular surface epithelium adjacent to the ventricles within the central nervous system, the neuroblasts that **divide symmetrically,** i.e., vertically, in the plane perpendicular to the ventricular surface, generate two equal daughter cells that have the capacity to divide further. However, the neuroblasts that **divide asymmetrically,** i.e., horizontally, in the plane

parallel to the ventricular surface, give rise to one neuroblast, capable of further cell divisions, and a more differentiated cell which can leave the cell cycle, migrate, and undergo terminal differentiation [4, 8].

Radial Versus Tangential Migration

Once a neural progenitor is generated via asymmetrical cell division, migration and terminal differentiation are frequently coupled. In general, there are two distinct modes of neuronal migration: radial migration and tangential migration. Excitatory neurons (expressing the neurotransmitter glutamate) usually migrate radially, whereas inhibitory interneurons (expressing the neurotransmitter GABA) often migrate tangentially, such as from the germinal matrix to the neocortex in humans and the ganglionic eminences to the neocortex in the mouse, where the basal forebrain is the primary source of GABAergic interneurons [9, 10].

Induced Pluripotent Stem Cells (iPSC)

Stem cells can self-renew through symmetric or asymmetric cell divisions (discussed earlier in this chapter). Several classes of stem cells have been described including **embryonic stem cells** (ESCs) and **induced pluripotent stem cells** (iPSCs). ESCs are derived from blastula's inner cell mass; they are **pluripotent** and can give rise to all differentiated cell types from the primary germ layers, the ectoderm, endoderm, and mesoderm. ESCs express several transcription factors, such as SOX2 and OCT-4, that repress differentiation. Although adult stem cells are relatively abundant in rapidly regenerating tissues, such as in the bone marrow and intestinal epithelium, there are "nests" of adult stem cells in the central nervous system and retina, in the subventricular zone and ciliary margins, respectively.

Due to ethical or practical limitations in place due to available sources of stem cells from the human embryo or adult, in the past decade, there has been significant interest in de-differentiating somatic cells such as epithelial cells and fibroblasts from adults into iPSCs. A few key master transcription factors, including OCT-3/4, SOX2, KLF4, and Nanog, have been identified that can reprogram differentiated cells into pluripotent cells and subsequently into specific neuronal populations. Furthermore, through viral and non-viral means, delivery of wild-type and edited genes through CRISPR/Cas9 technologies into iPSCs has the potential to treat many human diseases in which cell regeneration may restore structure and/or function, including neurodevelopmental disorders. Alternatively, these modified iPSCs can be screened for responses to chemical libraries toward identifying novel therapies [5, 11, 12].

Three-Dimensional (3D) Central Nervous System Organoids

More recently, there has been tremendous interest in modeling human brain development beyond the use of the commonly employed two-dimensional (2D) monolayer primary cell cultures *in vitro* or through the study of model organisms, including the zebrafish and mouse *in vivo*. Technological improvements (including spinner-flask bioreactors) and the advent of single-cell RNA sequencing have validated the diversity of cell types that can be generated from self-organizing, polarized, three-dimensional (3D) human brain organoids and their relative fidelity to the endogenous developing and adult brain with high organoid-to-organoid reproducibility. Furthermore, these models permit assessment of specific neuroanatomical regions (forebrain, midbrain, cerebellum, spinal cord, etc.), spatial organization, and cell–cell interactions including with the microenvironment. For example, using embryoid bodies, the addition of TGFβ inhibitors blocks mesendoderm lineage specification and promotes forebrain identity. BMP inhibitors block non-neural ectoderm lineage specification and promote dorsal forebrain identity. WNT inhibitors block both non-neural ectoderm and mesoderm lineages and promote forebrain identity [13].

There remain several limitations to 3D brain organoid systems, including an inability to fully replicate defined anatomical structures (such as the six-layer neocortex), missing cell types (e.g., microglia), absent vasculature, and the lack of functional neuronal networks. Recent innovations include co-culture with absent cell populations, providing an exogenous vascular supply and generating chimeric organoids from the combination of organoids from different brain regions. However, as experimental models, these 3D brain organoids provide a novel means to study normal and abnormal human brain development *in vitro*, thereby complementing studies in intact animal models and in tissues obtained from patients [13–15].

Disorders of Neural Induction, Early Regionalization, and Patterning

Holoprosencephaly

Holoprosencephaly (HPE) is a severe congenital brain malformation arising as a disorder of neural induction and regionalization with incomplete separation of the forebrain (prosencephalon). Five main types of HPE have been described (from severe to mild): (1) alobar; (2) semi-lobar; (3) lobar; (4) MIHV; and (5) microform. Its most severe phenotype includes complete lack of interhemispheric separation, a single midline forebrain ventricle, nonseparation of

deep gray nuclei and is frequently accompanied by cyclopia and severe craniofacial abnormalities. At the other end of the spectrum, there may be abnormalities of the corpus callosum and milder craniofacial anomalies observed, such as hypotelorism, coloboma, or cleft lip/palate. Neurocognitive impairment, feeding difficulties, seizures, and neuroendocrine abnormalities may be present and assessment by a multidisciplinary team as well as referral for genetic counseling is recommended.

Although holoprosencephaly can affect up to 1 in 250 conceptions, it is prevalent in only 1 on 10,000 live-born children. The etiology of HPE is very heterogeneous; HPE can occur as a single congenital disorder, as part of a syndrome (i.e., Smith–Lemli–Opitz or Kallmann syndromes) or a significant cytogenetic anomaly, including Trisomy 13. With the advent and availability of next-generation sequencing, mutations of several genes have been identified, including *SHH*, *TGIF1*, *FGFR1,* and the transcription factors *ZIC2* and *SIX3*. Other causes of HPE include submicroscopic chromosomal alterations and possibly to environmental influences, including maternal diabetes mellitus [16–19].

Anencephaly and Other Neural Tube Defects

Neural tube defects (NTD) arise due to failure of closure of the neural tube and occur in approximately 1 in 1000 live births worldwide [20]. NTD can occur anywhere along the rostral-caudal neuraxis and include disorders such as anencephaly (most anterior) to spina bifida (more posterior) and their variants. Although the majority of NTD occur as isolated congenital malformations, some are associated with syndromes and may have co-morbidities such as hydrocephalus and Chiari Malformations. The process of closure of the neural tube is discontinuous and occurs in the dorsal midline centered along three neuropores, which are open regions of neural folds: (1) hindbrain, (2) anterior (forebrain), and (3) posterior (spine). NTD can be open (anencephaly, craniorachischisis, or myelomeningocele) or closed, i.e., covered by epidermis (spinal dysraphism, spinal bifida occulta). *Primary neurulation defects* include craniorachischisis (18 days post fertilization/dpf), anencephaly (24 dpf), or open spina bifida (24 dpf). *Secondary neurulation defects* may be due to secondary neural tube tethering and can result in clinical disorders such as tethering of the spinal cord or spinal dysraphism with lipoma (35 dpf). *Postneurulation defects* include defects in skull closure, such as an occipital encephalocele with secondary herniation of the hindbrain and meninges (~ 4 months post fertilization) [1].

The causes of NTD can be genetic, environmental, or both. Closure of the neural tube has been studied in several vertebrate model systems. There is consensus that the process of **convergent extension** with convergence (mediolateral narrowing) and rostral-caudal extension is necessary. This requires the non-canonical Wnt signaling pathway via Frizzled (Fzd) membrane receptors and cytoplasmic Dishevelled (Dvl) to regulate epithelial planar cell polarity (PCP) processes. NTD can also result from dysregulation of bending of the neural folds at the median or dorsolateral hinge points of the primary neural tube. The Shh and BMP/TGFβ signaling pathways regulate these processes. Furthermore, NTD can be caused by full or partial failure of adhesion and fusion of the neural folds, experimentally supported by knockout mouse models in *ephrin-A5* or *EphA7* mutants [21]. Finally, other research has demonstrated that disordered cell proliferation and/or cell death can lead to NTD in experimental models (reviewed in [1]).

Although the majority of NTD occur sporadically, dozens of candidate genes have been implicated, often through the initial identification of NTD in single- or double-gene knockouts in the mouse model. NTDs can also be induced by **teratogens**, including the anticonvulsant medication valproic acid, which is also a histone deacetylase (HDAC) inhibitor. Various maternal risk factors include maternal fever/hyperthermia, obesity, diabetes mellitus, and nutrition during pregnancy [20]. Of significance, deficiency of the B-vitamin folic acid (folate) has been directly linked to the incidence of NTD. Clinical trials focused on primary prevention of NTD have demonstrated significant reduction in the occurrence of NTD in mothers who received folic acid supplementation. Most developed nations routinely supplement folic acid and maternal folic acid is a standard part of prenatal care. Although the mechanism linking maternal folate deficiency and NTD is not fully elucidated, it may include DNA methylation as a requirement for closure of the neural tube, as shown in *Dnmt3b* knockout mice [22].

Lissencephaly, a Neuronal Migration Disorder

Although there are many types of malformations of cortical development (MCD) with abnormal neuronal migration, this section will focus on lissencephaly (LIS). As classified [23], disorders of neuronal migration can be grouped as follows: (1) classic lissencephaly spectrum (includes smooth lissencephaly, microlissencephaly, and subcortical band heterotopia (SBH)); (2) cobblestone malformations (rough lissencephaly, polymicrogyria, leptomeningeal glioneuronal heterotopia); (3) periventricular heterotopia (nodular or linear periventricular heterotopia); or (4) dyslamination without cytologic dysplasia or growth abnormality (focal cortical dysplasia type I/FCD-I) [23]. Many patients with lissencephaly have epilepsy [24].

Classic lissencephaly (LIS) is relatively rare; morphologically there is *agyria* (absent cortical gyri) or *pachygyria* (very wide gyri) accompanied by a thickened cortical plate,

ectopic/displaced subcortical neurons and/or band/nodular heterotopias. Although LIS is usually an isolated cortical malformation, it may be part of a syndrome, such as Miller-Dieker and XLAG (X-linked LIS with ambiguous genitalia) often due to mutations of *ARX*, a transcription factor). Mutations of genes encoding cytoskeletal proteins have been implicated in classic LIS, whereas variant LIS may be linked to mutations of *REELIN* encoding a secreted protein, or other genes. *LIS1* (also known as *PAFAH1B1*, platelet-activating factor acetylhydrolase 1B) is located on chromosome 17p13.3; *LIS1* mutations are linked to classic LIS alone or as part of a chromosomal microdeletion in Miller-Dieker syndrome [25]. In part, *LIS1* encodes a cytoskeletal protein that interacts with microtubule associated proteins such as dynein required for neuronal migration. SBH is linked to mutations in *DCX* (doublecortin) located on chromosome Xq22.3-q23, encoding another microtubule associated protein [23]. Recently, several cytoskeletal disorders have been grouped together as *tubulinopathies*. Many tubulin gene disorders such as mutations of *TUBA1A*, are linked to severe malformations of cerebral cortical development, including lissencephaly and its variants.

Cobblestone LIS is due to histological defects linking radial glia (which support neuronal migration) to the basement membrane and results in dysregulated migration of neurons and glia into the subarachnoid space. Cobblestone LIS may be associated with CNS, muscular and/or ocular defects. Associated syndromes include Walker–Warburg syndrome, Muscle–Eye–Brain Disease and Fukuyama congenital muscular dystrophy (FCMD). Many of the genes associated with cobblestone LIS are part of the *α-dystroglycanopathies*, including *POMT1/POMT2*, *POMGNT1*, *FKTN*, *FKRP,* and *LARGE*. Other cases of cobblestone LIS are due to mutations of genes encoding laminins (*LAMB1/B2/C3*) [23, 26].

Hydrocephalus

Hydrocephalus is a relatively common disorder in children and sometimes occurs in adults. It can frequently accompany a closed NTD. When meningitis was a more frequently encountered disease of childhood, *communicating hydrocephalus* was a sequela of decreased reabsorption of cerebrospinal fluid (CSF). *Obstructive hydrocephalus* is often due to tumors of the CNS which frequently block CSF flow within or extrinsic to the ventricular system. In this section, the focus is on genetic disorders or syndromes for which congenital hydrocephalus is a major presenting sign. *X-linked hydrocephalus associated with stenosis of the aqueduct of Sylvius* (HSAS) is frequently due to mutations of the *L1CAM* gene encoding an adhesion molecule. Associated comorbidities may include agenesis of the corpus callosum, adducted thumbs, and X-linked spastic paraplegia. Other gene mutations resulting in congenital hydrocephalus occur in the *AP1S2* gene associated with X-linked intellectual disability and Fried syndrome with calcification of the basal ganglia, and in genes linked to α-dystroglycanopathies and cobblestone LIS briefly discussed in the preceding section [27]. Non-syndromic AR hydrocephalus is linked to mutations of the *CCD88C* and *MPDZ* genes, whereas hydrocephalus associated with the VACTERL (vertebral, anal, cardiac, tracheoesophageal, renal and limb anomalies) sequence has been linked to *PTEN* and *FANCB* (X-linked) [28].

Multiple Choice Questions

1. Which of the following overlapping stages of central nervous system (CNS) development is in the **INCORRECT** order?
   A. Induction of the neural plate
   B. Regionalization and patterning of the neural tube
   C. Migration of neurons
   D. Reflexes and behaviors
   E. Synapse formation
2. During development of the neural tube, what is the effect of **HIGHER** concentrations of retinoic acid above physiological levels?
   A. Anteriorization
   B. Dorsalization
   C. Posteriorization
   D. Ventralization
   E. Polarization
3. Which statement about cortical neurogenesis is **CORRECT**?
   A. Migrating cells result from asymmetrical cell division, perpendicular to the ventricular surface
   B. Migrating cells result from symmetrical cell division, perpendicular to the ventricular surface
   C. Migrating cells result from asymmetrical cell division, parallel to the ventricular surface
   D. Migrating cells result from symmetrical cell division, parallel to the ventricular surface
   E. None of the above
4. Which class of developing cells in the central nervous system rely on **TANGENTIAL** migration to reach their final destination in the cortex?
   A. Glutamatergic neurons
   B. GABAergic neurons
   C. Interneurons
   D. Radial glia
   E. B and C

5. Of the following genes, which one is **NOT** associated with holoprosencephaly:
   A. SHH
   B. PTEN
   C. SIX3
   D. FGFR1
   E. ZIC2

   Answers: 1D; 2C; 3C; 4E; 5B.

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Neural Stem Cells, Differentiation, and Migration

3

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Highlights

- Neural stem cells build the central nervous system during development, and persist in the adult.
- Neural stem cells demonstrate heterogeneity across age and sex.
- Neural stem cells can contribute to neural repair following injury and disease.
- Cerebral organoids provide a novel tool for examining human neural precursor populations in health and disease.

Learning Objectives

- Understand the fundamental biology of neural stem cell populations in the developing and mature central nervous system
- Know where neural stem cells reside in the central nervous system
- Identify the role of the stem cell niche in modulating cell behaviour, and how this niche changes with regard to sex, age and injury
- Become familiar with approaches to using stem cells for neural repair
- Understand the challenges associated with neural stem cell-based therapeutic approaches

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Introduction

'Once development was ended, the founts of growth and regeneration...dried up irrevocably. In adult centres the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated' [1]. This famous passage written in 1928 by Santiago Ramón y Cajal, the father of modern neuroscience, remained a dogma in the study of the central nervous system (CNS) for decades. This view was first challenged in 1961, when Smart and Leblond used tritiated thymidine to demonstrate cell proliferation in the uninjured post-developmental CNS [2]. Altman and colleagues further demonstrated that proliferating cells were present in the periventricular region lining the lateral ventricles in the forebrain and in the olfactory bulb (OB) [3]. In the following decade, Reynolds and Weiss made a seminal breakthrough, demonstrating that the post-developmental mammalian CNS contained a population of neural stem cells (NSCs) [4]. These cells were identified as rare, slowly dividing subependymal cells in the adult forebrain [5] and later they were also identified in the periventricular region along the length of the spinal cord [6]. Concurrently, the dentate gyrus (DG) of the hippocampus was identified as a neurogenic region in the adult brain [7], and studies in the 1990s revealed that the DG also contains a population of NSCs [8].

Here, we discuss NSCs and their progeny, collectively termed 'neural stem and progenitor cells' (NSPCs). We examine their developmental origin and lineage dynamics through development and into the mature CNS. We discuss recent work examining NSC heterogeneity, and the resident niches that regulate NSC behaviour, under physiological conditions and in the event of injury or disease. Finally, we summarize ongoing approaches and scientific innovations that have the potential to expand our knowledge of NSCs and harness their properties to treat the injured and diseased CNS.

Over the last several decades, significant advances have been made in our understanding of mammalian CNS development. Studies investigating the cellular and molecular events that underpin the emergence of the intricate and

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dynamic structure of the CNS reveal the importance of NSCs during development and throughout the lifespan. This chapter will review the role of NSCs in building the CNS from its early embryonic state through development and highlight key features of the developing and mature niche that regulate their behaviour. Much of the research discussed will focus on work performed using rodent models, with some human comparisons, including *in vitro* models of human brain development.

Building the Central Nervous System

It is well established that NSCs are the fundamental building blocks of the CNS, generating all neural cell types; neurons, astrocytes, oligodendrocytes, and most recently, they have also been shown to generate ependymal cells (multi-ciliated epithelial cells that line the ventricular system) [9]. Additional non-neural cells including microglia, vascular endothelial cells and pericytes also exist in CNS and play crucial roles in regulating NSC behaviour, but these cells do not originate from NSCs. During development and in the mature CNS, NSCs are confined to specific 'neurogenic niches' and arise from the periventricular zone (PVZ) along the neuroaxis.

Development of the CNS begins early in embryogenesis (human embryonic [E] day 13 of the 280 days gestation period; E4.5 of the 21 day gestation period in mice), when the embryo is a two-layered structure, containing an upper 'epiblast' cell layer which gives rise to the embryo proper, and a lower 'hypoblast' cell layer which forms the extraembryonic tissues (e.g. the placenta) [10]. The embryo proper dramatically changes shape through a process known as gastrulation (human E15; E6 in mice), establishing the three primary stem cell populations from the epiblast: ectoderm, mesoderm and endoderm [10]. Each of these stem cell populations goes on to give rise to specific structures within the developing embryo.

During the earliest stages of CNS development, a conserved molecular signalling system polarizes the embryonic ectoderm into the neuroectoderm. During this process, Wnt signalling mediates ectodermal specification [11]. Signaling by anti-neurogenic bone morphogenic protein (BMP) effectively represses neural identity, driving the formation of non-neural ectoderm. Conversely, the absence of BMP, in combination with the presence of fibroblast growth factor (FGF), directs the formation of the neuroectoderm [11]. Once the neuroectoderm is specified, a second set of conserved developmental control genes is activated. These are pro-neural genes that encode basic helix–loop–helix (bHLH) transcriptional factors including Mash1, Neurogenin 1 (Ngn1), and Neurogenin 2 (Ngn2), which promote the transition of neuroectodermal cells into NSCs [12].

The resulting sheet of neuroectodermal cells comprises the neural plate, which then undergoes a process known as neurulation to form the neural tube (human E28; E9.5 in mice). This structure represents the embryonic precursor to the mature CNS. Neural tube formation begins with the appearance of two ridges on the lateral edges of the neural plate. Over the course of several days, these ridges rise upwards, fold together and eventually fuse in the midline to form the neural tube. Hence, in its most primitive form, the neural tube consists of a single layer of neuroectodermal cells that surrounds a hollow centre, creating a primitive ventricular system [10]. Through development, morphological changes occur such that the rostral end of the neural tube changes shape to form three primary brain vesicles: the prosencephalon (forebrain), mesencephalon (midbrain) and rhombencephalon (hindbrain) [10]. These three regions further subdivide into five secondary vesicles; the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon, which collectively form the foundation of the mature brain. The caudal neural tube gives rise to the spinal cord.

Embryonic Neural Stem Cells, Migration, and Differentiation

The CNS derives from the proliferation of neuroepithelial cells comprising of the neural tube. While little is known about the heterogeneity of neuroepithelial cells, it is at these very early times in CNS development that a population of rare, 'primitive' neural stem cells (pNSC) are first observed (as early as E5.5 in mice) [13]. pNSCs are multipotent cells that can be isolated from the neural plate; they express the pluripotency marker Oct4 and are responsive to leukemia inhibitory factor (LIF) [14, 15]. Intriguingly, pNSCs persist in rare numbers throughout development and into the mature and aged CNS [14, 16]. It is proposed that pNSCs are a distinct subpopulation of neuroepithelial cells [17]. Compelling lineage tracking studies reveal that pNSCs are multipotent during development, contributing to neural cells throughout the parenchyma, and can also be activated in response to neural injury [15] (see Box 3.1 for details).

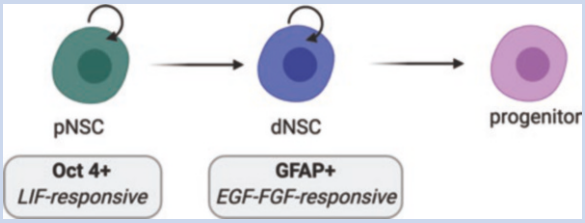
Box 3.1 Uncovering the Potential of Rare Stem Cell Populations

Primitive neural stem cells (pNSCs) are a novel stem cell population that first appear in the developing embryo of mice at E5.5 [13]. These cells persist following development, though they are extremely rare in the postnatal CNS11. To date, pNSCs have been identified in the SEZ and the PVZ but have not been isolated from the SGZ.

pNSCs express the pluripotency marker Oct4 and are responsive to leukemia inhibitory factor (LIF) [14]. They express ErbB2 and c-Kit receptors [17] making them distinct from definitive NSC (dNSCs). Moreover, pNSCs do not express the dNSC marker, GFAP. Similar to dNSCs, pNSCs are injury-responsive, expanding in number following CNS injury. Lineage tracking experiments have established a

hierarchical relationship between pNSCs and dNSCs throughout development [14] and reveal the multipotent, self-renewing capabilities of pNSCs *in vivo*, with the generation of neurons, astrocytes, and oligodendrocytes during embryogenesis. Further, pNSC transplantation and adult lineage tracking studies reveal that pNSCs contribute to ongoing adult neurogenesis and are the source of dNSC repopulation in models of stem cell ablation [14]. These studies highlight the potential for pNSCs to serve as a source of cells for regenerative strategies to improve neurorepair.

The study of pNSCs poses a number of unique challenges. First, they are identified based on their expression of Oct4 which is a marker of pluripotent cells and they arise at the onset of neurulation when Oct4 expression persists throughout the developing neural plate making them challenging to delineate at early timepoints [13]. It has been proposed that pNSCs are a subpopulation of neuroepithelial cells [101] but the precise relationship between pNSCs and neuroepithelial cells, as well as RG, remains unclear. Further, because they are exceedingly rare and have a very long cell cycle time (3–5 months) [101] in the post-developmental CNS, it is challenging to analyse their lineage dynamics. Ongoing studies are focused on elucidating these details and continue to explore their frequency, genetic profile, cell cycle kinetics, and differentiation potential to explore their regenerative capacity. *Created with BioRender.com*.



As the CNS continues to grow in size, neuroepithelial cells proliferate rapidly through a series of symmetric divisions, effectively expanding the size of the stem cell pool (Fig. 3.1) and give rise to radial glial (RG) cells (Fig. 3.2). RG have a soma in the periventricular region, and a basal process extend-

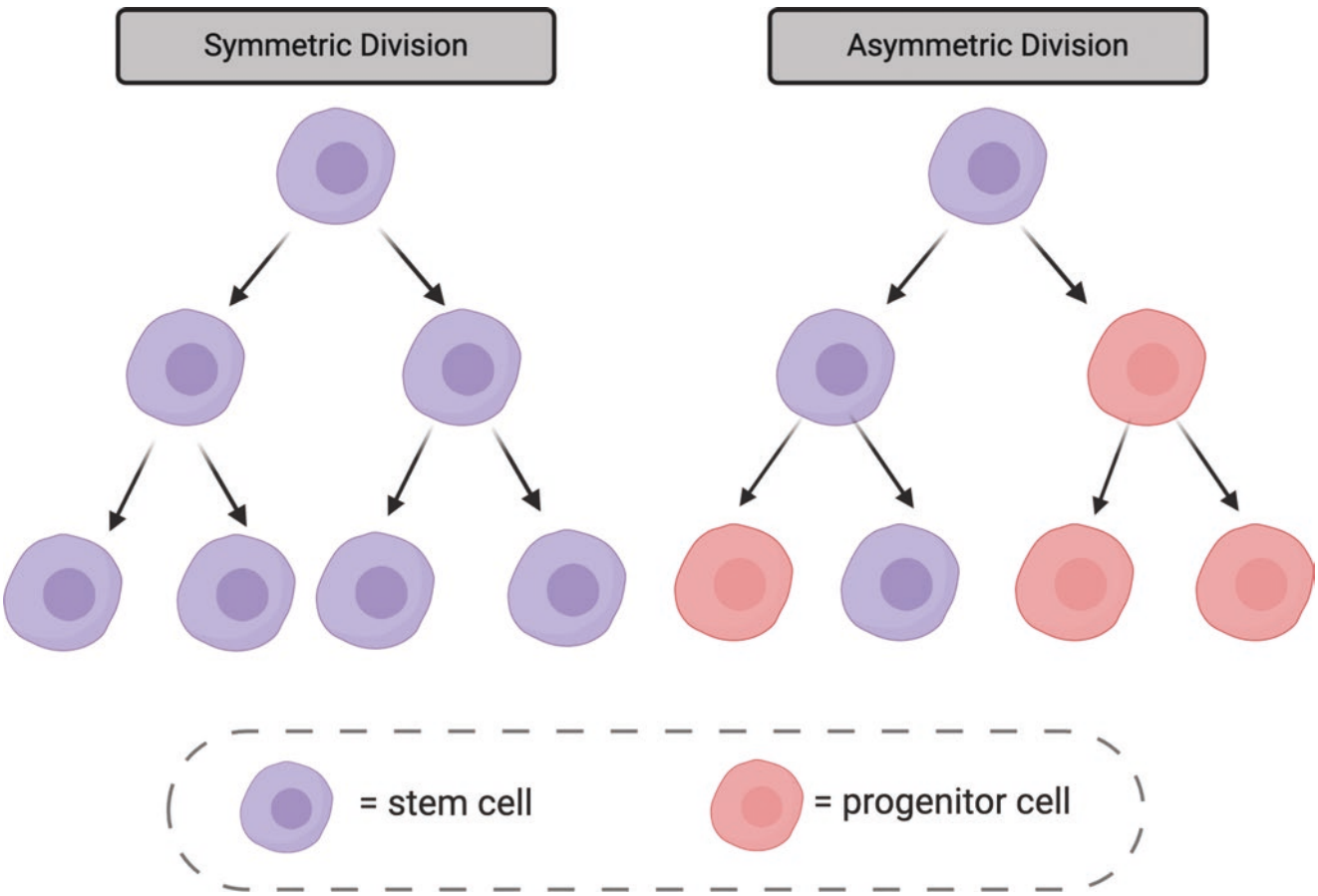


Fig. 3.1 Modes of stem cell division. Neural stem cells can undergo asymmetric or symmetric divisions. In symmetric division, a stem cell produces two copies of itself (thereby expanding the size of the stem cell pool). In asymmetric division, the stem cell self-renews to produce a copy of itself (to maintain the size of the precursor cell pool), and one progenitor. The progenitor has a limited capacity for self-renewal and will generate differentiated progeny through additional asymmetric or terminal (differentiation) divisions. *Created with* BioRender.com

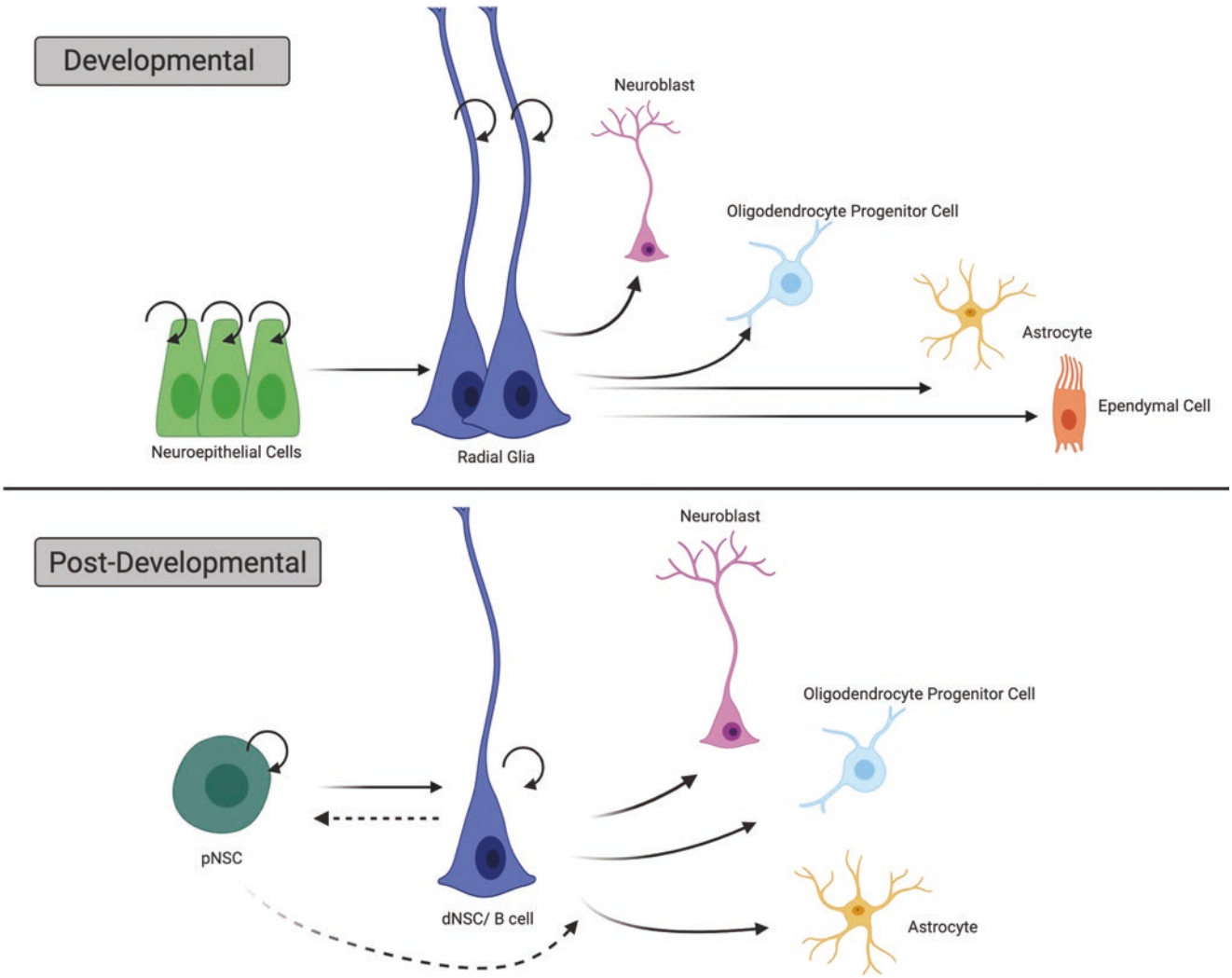


Fig. 3.2 Neural development. During development, neuroepithelial cells generate radial glia, which are multipotent cells capable of generating all neural cell types. This process is temporally regulated, with radial glia serving as a source of stem cells that first give rise to neuroblasts (neuronal precursors), then later give rise to glial progenitor cells (which go on to produce mature oligodendrocytes and astrocytes). Through development, a subpopulation of radial glia will terminally differentiate as ependymal cells that line the periventricular system and a subpopulation persist into the post-developmental period as bonafide NSCs in specific neurogenic niches. In the post-natal CNS, a rare population of primitive NSCs (pNSC) have been identified. Their precise role in adult neurogenesis remains unknown (see Box 3.1); however, there is compelling evidence that they can self-renew to produce 'definitive' NSCs (dNSCs). Whether they can contribute directly to differentiated progeny, without going through a dNSC state, is an interesting question under investigation. dNSCs in the adult brain can give rise to neurons, oligodendrocytes, and astrocytes. *Created with* BioRender.com

ing to the outer (pial) surface of the developing CNS [18, 19]. Originally identified as the cells responsible for guiding cell migration during development [19], it is now clear that RG comprise a subpopulation of NSCs [19, 20]. RG can be identified by their expression of brain-lipid-binding-protein (BLBP), radial cell 1/2 (RC1/2), and the glutamate transporter GLAST. Virtually all RG divide during development and have the capacity generate neurons and glia [18]. In the maturing brain and into adulthood, RG retain their location within the periventricular region, retract their basal process from the pial surface, and serve as adult NSCs [20].

The generation of neural cell types in the developing CNS occurs in a step-wise fashion that begins with neurogenesis. Cortical neurogenesis begins at E11.5 in mice (gestation week 6 in humans) with the earliest born neurons comprising the deep layers of cortex, and later-born neurons occupying more superficial locations near the pial surface [21]. The earliest newborn neurons migrate through a process known as somal translocation, wherein they extend a process to the pial surface, following which the nucleus moves to the cortical location. As the brain expands, so does the distance neurons need to travel, and the newborn cells begin to use RG as scaffolds to enable rapid, region-specific migration [19]. The fate of the newly generated neurons relies on environmental factors in which the RG resides [21].

The switch from neurogenesis to gliogenesis (oligodendrocytes and astrocytes) occurs at ~E16.5 in the mouse (gestational week 24 in humans), peaks around birth, and

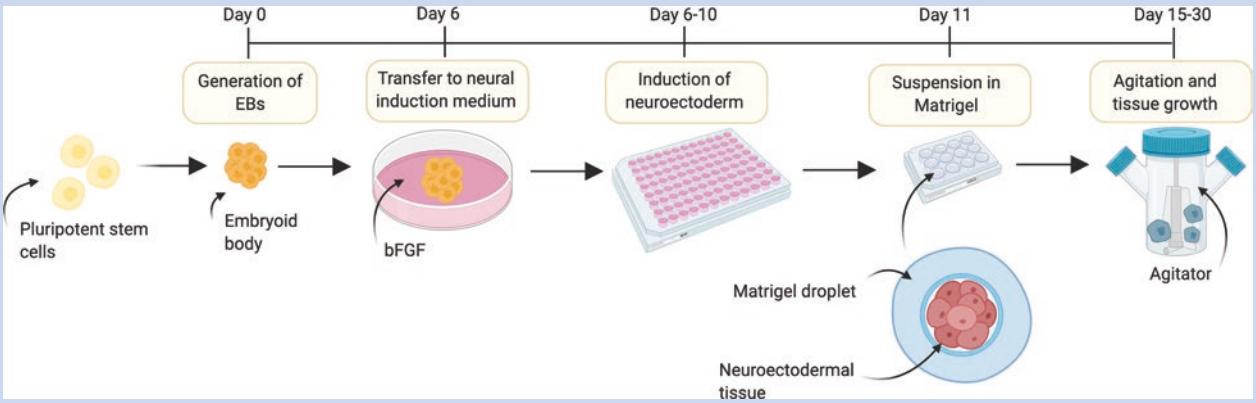
continues into the postnatal period [22]. Gliogenesis involves the activation of the JAK-STAT, BMP, and Notch signalling pathways [21]. Glial cells are generated from RG born in specific areas of the ventral neural tube with intermediate oligodendrocyte progenitor cells (OPCs) arising first. Oligodendrogenesis to myelinate axons occurs in the early postnatal period, and a subpopulation of OPCs persists within the parenchyma that can give rise to oligodendrocytes throughout the lifespan. Astrocyte production begins in the late embryonic period [10]. Interestingly, despite compelling evidence that astrocytes are a diverse population of cells, little is known about the molecular and ontogenetic origin of these cells. Evidence from studies in spinal cord development reveals that RG location plays a role in the generation of unique astrocyte subpopulations. These regionally specified subpopulations can be delineated based on the expression of transcription factors including PAX6, Reelin, and Slit1 [23]. Future studies will undoubtedly focus on the developmental regulation of the diversity of astrocytes, the most abundant neural cell type in the CNS. Finally, in the late embryonic stage, following the period of gliogenesis, individual RG can give rise to mitotically quiescent, multiciliated, ependymal cells [9] or persist as NSCs that contribute to neurogenesis in the mature brain [20] (Fig. 3.2).

Recent advances in stem cell biology have provided a means to study human neural development using novel *in vitro* techniques. Given the complexity of the human brain, finding an approach to recapitulate all of developmental characteristics is challenging. The establishment of novel human-derived organoid cultures has enabled the *in vitro* study of CNS development and the role of NSCs (highlighted in Box 3.2). Grown from human-derived pluripotent embryonic stem cells, cerebral organoids represent a three-dimensional model capable of capturing characteristics of

Box 3.2 Cerebral Organoids

Cerebral organoids can be grown from pluripotent stem cells *in vitro* [24]. They self-organize into complex brain-like structures with multiple distinct and interconnected tissue regions. Cerebral organoid cultures were originally described in 2013, and since then, optimization of culture conditions has allowed for the growth of specific brain areas [24]. This provides the ability to recapitulate characteristics of cortical organization not seen in *in vivo* models, including spatial organization of neuronal subtypes into discrete layers, spontaneous development of signalling centres to dictate tissue patterning, and recapitulation of the gyrencephalic nature of human brains. In addition, they can be grown directly from patient-specific cells, allowing for the comprehensive study of various disease-specific phenotypes. Excitingly, a number of studies have used cerebral organoids to model phenomena not previously possible *in vitro*. For example, organoids have been used to model microcephaly using patient-derived stem cells. These studies revealed evidence of premature neuronal differentiation, a defect that could explain the reduced brain size resulting from that condition.

Currently, the use of cerebral organoids has limitations, and optimization of cortical organoids continues to evolve. To date, cerebral organoids only produce cells of neuroectodermal descent, so vascular systems and cells such as microglia are not present. Considering the significant contributions of non-neural cell types during the complex stages of brain development and their role in the NSPC niche, cerebral organoids are limited in some aspects beyond early developmental stages. The lack of vascular systems also imposes size limitations; most cerebral organoids grow to just a few millimetres in size before the cells deep in the structure die due to lack of oxygen and nutrients reaching their centre [24]. Additionally, the degree of mature cortical layer organization observed *in vitro* is less complex than that seen in the brain, suggesting the need for additional cues [24]. Nonetheless, cerebral organoid culture is an exciting and novel technique that can be applied to model early brain development, and their application continues to progress as further studies aim to establish methods to provide circulation and other signalling mechanisms. *Created with BioRender.com*.



brain development not possible with traditional two-dimensional *in vitro* approaches [24]. Cerebral organoids consist of multiple distinct and interconnected regions, with signalling centers that recapitulate developmental tissue patterning [24]. Gene expression in cerebral organoids is remarkably similar to that of the fetal neocortex, and the electrophysiological activity of organoids reflects that of the human brain [25].

Neural Stem Cell Heterogeneity

Following development, NSCs persist in specific periventricular regions of the mature CNS: the subependymal zone (SEZ) lining the lateral ventricles, the subgranular zone (SGZ) of the hippocampus, and the PVZ lining the central canal of the spinal cord (Fig. 3.3). Some studies have reported the identification of NSCs in other regions of the brain including the cortex, striatum, hypothalamus, and substantia nigra [26]; however, validation of these less well-delineated NSC populations is needed and will not be the focus of this chapter.

NSCs are identified within the CNS using a combination of cell morphology, mitotic activity, and cellular markers. There is no single marker that enables the unequivocal identification of NSCs; however, a panel of markers used in their identification include the transcription factor Sox2; intermediate filament proteins Nestin and glial fibrillary acid protein (GFAP); Musashi-1; CD133; epidermal growth factor receptor (EGFR); and Oct4. Similar to stem cells from other tissues, NSCs can be identified retrospectively using *in vitro* assays. The neurosphere assay and adherent monolayer cultures, described in detail in Box 3.3, are most commonly used for the isolation and characterization of NSCs.

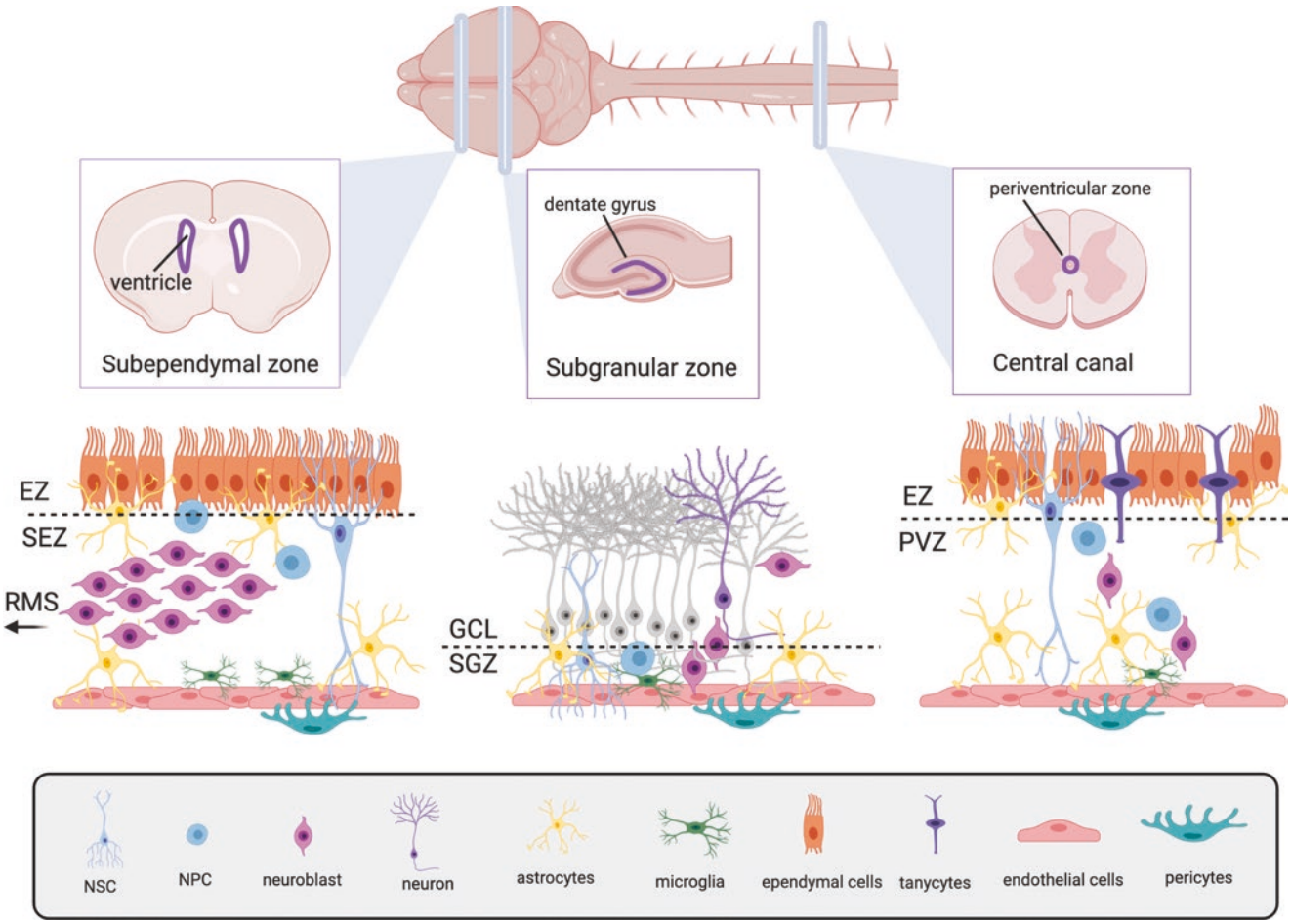
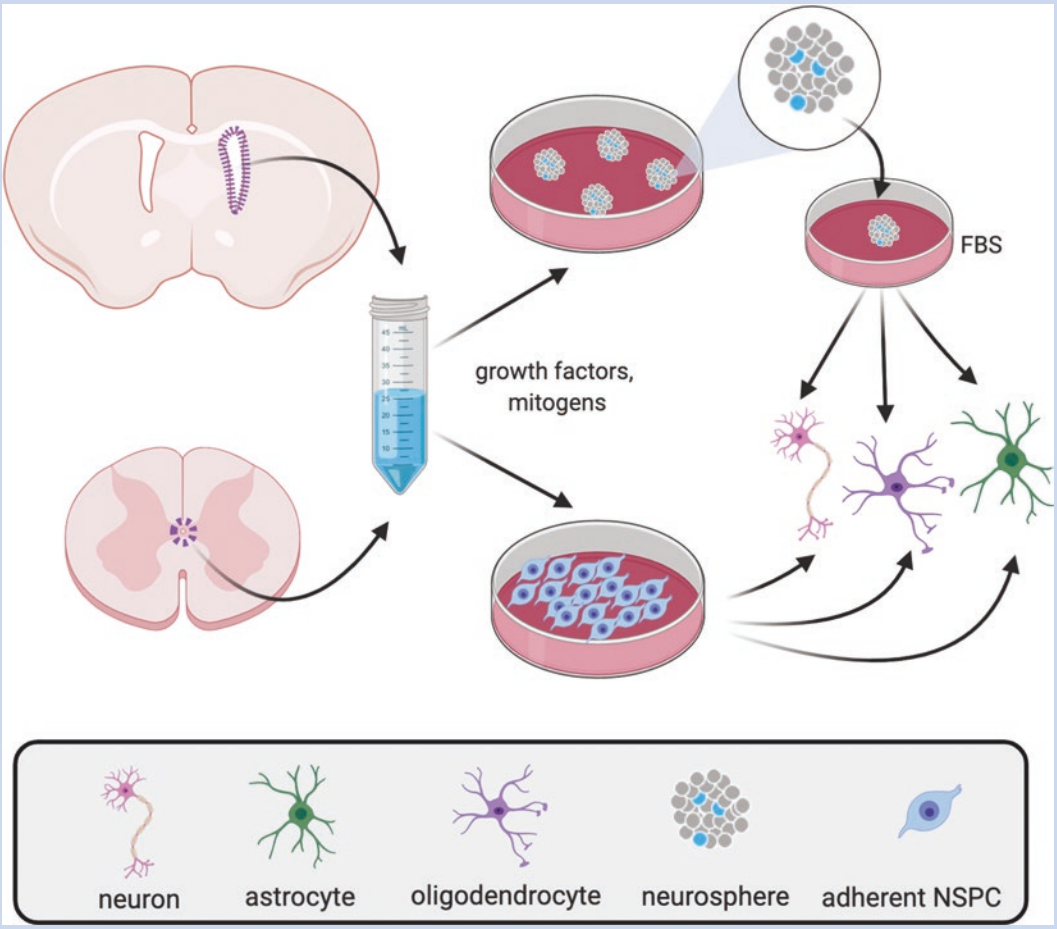


Fig. 3.3 Neural stem cell niches in the post-developmental CNS. In the postnatal brain, there are two niches that contain NSCs that contribute to ongoing neurogenesis. The SEZ lining the forebrain lateral ventricles generates neuroblasts that migrate along the rostral migratory stream to the olfactory bulb, where they mature into interneurons that participate in olfaction. The SEZ-NSCs also give rise to oligodendrocytes within the corpus callosum under baseline conditions. A second neurogenic niche is the dentate gyrus of the hippocampus, where NSCs in the SGZ generate neurons that migrate radially into the granular cell layer and generate neurons that participate in memory function. A smaller population of NSCs reside in the spinal cord within the PVZ lining the central canal. Under homeostatic conditions, the spinal cord is aneurogenic, and NSPCs are primarily mitotically quiescent. Each stem cell-containing niche is distinct, and comprised of various neural and non-neural cell types, as well as signalling molecules and extracellular matrix that profoundly affects NSC behaviour. *Created with* BioRender.com

Box 3.3 Identifying Neural Stem Cells *In Vitro*

The neurosphere assay was originally described in 1992 by Reynolds and Weiss [4]. This *in vitro* colony forming assay demonstrates the two cardinal properties of stem cells: self-renewal and multipotency. It is a simple and robust assay to assess NSPC behaviour when used with a sound knowledge of its limitations. The assay involves the microdissection of NSPCs from the brain or spinal cord, and dissociating and plating them *in vitro* in the presence of mitogens including fibroblast growth factor (FGF) and epidermal growth factor (EGF) for 7–10 days [4]. When plated at clonal density, NSCs proliferate to form free-floating spherical colonies known as 'neurospheres'. Colonies that are >80 um in diameter are comprised of multipotent NSCs and their progeny. Individual neurospheres consist of hundreds of cells, of which a small percentage (1–5%) are stem cells, and the vast majority are progenitor cells. As such, the number of neurospheres indicates the size of the NSC pool, while the size of individual neurospheres reflects the proliferative capacity of the progenitors. Self-renewal is demonstrated by selecting, dissociating and re-plating cells from individual neurospheres (termed 'passaging'). Since each neurosphere is derived from a single NSC, the numbers of secondary neurospheres reflect the number of NSCs that were present within the growing colony. Multipotency can be investigated by placing individual neurospheres in differentiation conditions (e.g. fetal bovine serum) and examining the cell phenotypes generated.

Stem cells can also be identified using *in vitro* adherent monolayer cultures**.** The dissection and dissociation process is similar to that used in the neurosphere assay [102]; however, rather than growing free-floating spherical cultures, this technique involves plating NSPCs as two-dimensional adherent cultures. Cells are plated in a culture dish for 24 h, following which all non-adherent cells are transferred and grown in the presence of EGF and FGF for 7 days [102]. Because this assay does not require clonal plating density, this system allows for cell–cell contact that may influence NSPC behaviour. This approach allows for the study of potential paracrine signalling effects, and circumvents the challenges associated with oxygen and supplement availability to the cells in the centre of neurospheres. However, adherent cultures are limited in their capacity to quantify and assess individual NSC behaviour. *Created with BioRender.com*.



Studying the kinetics of post-developmental NSCs has revealed heterogeneity within this population. NSCs exist in two states: activated and quiescent. In their activated state, NSCs proliferate and are active in protein translation and synthesis. In their quiescent state, they are non-proliferative, and protein synthesis is relatively low or absent [27]. Activated and quiescent NSCs can be identified based on their unique molecular profiles, whereby activated NSCs express high levels of Nestin and EGFR and quiescent NSCs have low Nestin expression and are EGFR negative [28]. Importantly, NSCs can switch between activated and quiescent states with the balance between these two states dependent on signalling, for example, via sphingosine-1-phosphate (S1P), prostaglandin D2 (PGD2), and Notch. With aging, there is a shift in the relative proportions of activated and quiescent NSCs, and this is thought to underlie decreased neurogenesis observed with age. Activation state can also be shifted by environmental stimuli such as injury or disease, during which there are upregulated signalling molecules including interferon gamma [27]. Modifying the ratio of quiescent to activated NSCs may provide novel mechanisms to slow down the effects of aging on neurogenesis, and to enhance neurorepair.

Neurogenic Niches

Neurogenic niches in the post-developmental CNS are complex, multi-cellular regions that support the production, division, and maintenance of NSPCs throughout life. The environments within which NSPCs reside are composed of a variety of cells, including endothelial cells, astrocytes, and microglia; are surrounded by the extracellular matrix (ECM); and are exposed to secreted and circulating factors and afferent projections from neurons that secrete neurotransmitters into the niche. These components underlie the complex interplay of signalling molecules and physical elements that modulate NSPC behaviour to maintain neurogenic homeostasis.

The Subependymal Zone

The SEZ is the largest neurogenic niche in the postnatal CNS. This region is derived from the germinal zone during embryogenesis and continues to generate new neurons and glia from resident NSCs after birth. SEZ-derived NSCs primarily generate neuroblasts through the process of asymmetric division (Fig. 3.1) that migrate along the rostral migratory stream (RMS) to the olfactory bulb where they differentiate into mature, functionally integrated interneurons [29, 30]. Neuroblasts migrate in aggregates of unipolar and bipolar cells, forming chain-like structures where they move alongside and over top of one another [31]. Migratory neuroblasts express polysialylated-neural cell adhesion molecule (PSA-NCAM) and the microtubule-associated protein doublecortin (DCX). Once they reach the olfactory bulb, neuroblasts exit the chain through interactions with ECM proteins (e.g. Reelin), migrating radially to the outer layer where they differentiate into interneurons in the granule or periglomerular layers [31]. Migratory guidance is orchestrated by various attractive cues, including morphogens (e.g. Sonic hedgehog), trophic factors, and ions, as well as repulsive cues along the RMS and within the cerebrospinal fluid (CSF) of the ventricular system (e.g. Slit2) and the direction of CSF flow [31]. Neuroblast migration occurs in close association with blood vessels and astrocytes that act as scaffolds to guide and facilitate migration. The ultimate survival and integration of migrating neuroblasts are dependent on glutamate released from astrocytes along the RMS [32].

The SEZ is adjacent to a single layer of ependymal cells, which separate NSCs from the ventricle. Ependymal cells are multiciliated, non-neural cells with apical processes that contact the CSF and form the epithelial lining of the ventricular system. They are connected to each other by tight junctions, and to astrocytes through gap junctions [33]. Ependymal cells produce signalling molecules such as Noggin, an antagonist to the neurogenesis-inhibiting morphogen BMP [34], which directly regulate neurogenesis. These cells have access to signalling molecules released by the choroid plexus into the CSF, which are known to regulate NSPC behaviour and neurogenesis in an age-dependent manner [35].

The neurovascular niche within the SEZ also plays a prominent role in regulation of NSPC behaviour. Vascular endothelial cells are in direct contact with the basal processes of NSCs. Endothelial cells provide nutrients and release modulating factors such as ephrin-B2 and neurotrophin-3 (NT3), both of which maintain NSCs in a quiescent state [36].

Microglia, the immune cells of the CNS, are found in the SEZ. These cells function to detect changes in homeostasis, and become activated during injury and/or inflammation, releasing cytokines. Microglia are critical for the regulation of NSPC proliferation, migration [37], and differentiation into mature cell phenotypes [38]. Depletion of microglia from the niche results in reduced levels of cytokine release and decreased neurogenesis and gliogenesis [39].

Regulation of NSPC behaviour also occurs via the ECM [40]. The most prominent components responsible for interactions between NSPCs and the ECM are integrins, which have been shown to regulate adhesion and anchoring of NSPCs and direct NSC self-renewal [41]. Further, the ECM regulates NSPC behaviour by controlling the accessibility of growth factors within the niche (for example VEGF, FGFs, BMPS) [40], binding or releasing them for presentation to NSPCs.

NSPC behaviour is also controlled by projections from neurons in remote areas of the brain. For example, proopi-

omelanocortin neurons from the hypothalamus innervate the SEZ and promote the proliferation of NSPCs [42]. Dopaminergic projections from the substantia nigra synapse with cells in the SEZ and the loss of this dopamine input, as would be seen in Parkinson's Disease for example, lead to a reduction in neurogenesis [43]. Brainstem projections from serotonergic neurons comprising the raphe nucleus synapse directly onto NSPCs and cause increased proliferation [44]. These long-distance projections from remote brain regions allow for the direct modulation of neurogenesis in response to physiological needs and in injury or disease states.

The Subgranular Zone

The SGZ is a region of the DG of the hippocampus that contains a population of NSCs that share many features with those of the SEZ. This zone is formed from a subset of precursor cells referred to as the dentate neuroepithelium (DNE), which appears between E9.5 and 12.5 in mice (gestation week 6 in humans) [45] and eventually comprise the NSCs of the adult SGZ. Hippocampal neurons first appear at E14.5 (gestation week 10 in humans) when the hippocampal fissure has formed.

NSCs in the SGZ are referred to as 'radial glia-like cells'. Under normal conditions, they generate neurogenic progenitor cells that migrate tangentially and then radially into the granule cell layer (GCL) of the hippocampus [46]. Similar to migratory cues for SEZ neuroblasts, progenitor cell migration occurs in response to extracellular cues including the ECM protein Reelin [47] and secreted factors from neighbouring cells. Migrating progenitor cells interact directly with vasculature [46] and other migrating cells [48], using them as scaffolding as they travel to their destination. Notch signalling controls the maintenance of stemness in SGZ NSCs, as well as migration and differentiation of the resulting progenitors [48]. A majority of progenitor cells undergo programmed cell death within 1–4 days of production and are cleared by microglia [37], while those that survive differentiate into mature neurons involved in learning and memory.

Similar to NSCs in the SEZ, NSCs in the DG express Sox2, Nestin, and GFAP, and exist in activated or quiescent states. Moreover, while the generation of neurons throughout life is common to both the SEZ and SGZ, the organization of the SGZ niche is starkly different from that of the SEZ. For instance, in the SGZ, astrocytes represent the principal cellular component [49]. Astrocytes modulate NSPC proliferation and differentiation through direct contact, as well as through the release of growth factors [49] and signalling molecules such as Notch and Wnt [50]. Further, SGZ astrocytes have fine, highly branching processes extending through the DG and into proximal regions of the structure [51], enabling NSPCs to have both direct and indirect contacts with various cells of the niche that can in turn regulate their behaviour [51].

Similar to the SEZ, NSPCs in the DG directly contact vascular endothelial cells [52] which impact NSPC behaviour. Interestingly, stimulation of blood vessel formation, termed 'angiogenesis', leads to increased neurogenesis in the DG. For example, exercise or exposure to complex environments that provide opportunities for physical and social enrichment [referred to as 'environmental enrichment' (EE)] [53] leads to increased release of growth factors including vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) in the DG, and increased angiogenesis and neurogenesis in this region [54]. Reducing neurotrophin signalling attenuates exercise-induced neurogenesis [54].

Microglia are also prominent within the SGZ [55]. Under normal conditions, these microglia phagocytose apoptotic NSPCs and other debris. In the event of inflammation, they can become activated and release pro-inflammatory cytokines (e.g. IL-1β, IL-6, and TNF-α) that limit neurogenesis [55]. Moreover, microglia can affect the differentiation profile of NSPCs depending on the particular profile of cytokines released [56].

Unlike the SEZ, there is limited evidence of remote innervation affecting NSPC behaviour in the DG. GABAergic interneurons innervate the hippocampus in regions including the SGZ, and inhibition of GABA signalling from these neurons may affect NSPC behaviour [57] .

The Periventricular Zone

The PVZ of the spinal cord is derived from the caudal neural tube, and within this region, the vast majority of NSCs reside in direct contact with the lumen of the central canal. NSCs in the spinal cord express GFAP, Sox2, and Nestin similar to those in the brain [58], and a subset express MSX1 [59]. Under homeostatic conditions, NSCs in the spinal cord exhibit slow cell cycles and do not contribute to ongoing neurogenesis; however, following injury, they become activated and can give rise to progenitor cells that migrate toward the damaged region, which primarily produce astrocytes and oligodendrocytes [58].

Within the spinal cord, the components and dynamics of the niche are less well delineated. Nonetheless, some similarities to brain are observed. The PVZ niche is a heterogeneous region composed of several different cell types positioned in highly specified domains that serve specific functions [59]. A majority of cells comprising the PVZ niche are multi-ciliated ependymal cells and tanycytes. Tanycytes are morphologically discernable from ependymal cells based

on the presence of a long basal process that connects them to surrounding blood vessels and are located in the dorsal and ventral regions of the niche. These cells transport signalling molecules between the vasculature and the niche, and serve as the primary link between the CSF, blood, and neuroendocrine factors [59]. The PVZ niche also contains a population of inhibitory neurons that are in direct contact with ependymal cells [60]. These neurons are largely thought to function as mechanoreceptors capable of sensing CSF flow and pressure [61].

Regulating the Neurogenic Niche: Sex, Age, and Injury

Considering the profound impact that the neurogenic niche has on NSPC behaviour, it is important to recognize factors that affect niche composition and signalling. These include sex, age, and injury or disease, which have been shown to directly and indirectly impact NSPC behaviour. Perhaps not surprising, it has been shown that different hormonal profiles in males and females affect NSPC dynamics. The presence of the female sex hormone estradiol increases stem cell proliferation and enhances neurogenesis [62, 63], in part due to different ratios of estrogen receptor subtypes expressed on the NSPCs of males and females [64]. Another example is the pregnancy hormone prolactin, which has been shown to regulate olfactory bulb neurogenesis [65], postulated to play a role in offspring recognition. Prolactin receptors were initially thought to be present on NSPCs; however, more recent studies have been unable to confirm this finding. As such, it is likely that the effects of prolactin are indirect and acting through changes to the niche to regulate NSPC behaviour. In line with this hypothesis, prolactin receptors are expressed by cells of the choroid plexus within the lateral ventricle [66]. The male sex hormone testosterone has an opposing, inhibitory effect on neurogenesis [67]. Indeed, removal of testosterone by castration leads to increased cell proliferation in the SEZ [63]. Further, sex-dependent differences in NSPCs appear to be regionally distinct. In the DG, testosterone does not affect cell proliferation but instead enhances NSPC survival, ultimately resulting in increased neurogenesis in the hippocampus [67]. This highlights the complexity of the mechanisms by which sex hormones can regulate NSPCs, and demonstrates that sex-dependent NSPC behaviour reflects hormonal dynamics across the lifespan [62].

The effects of aging on NSPC behaviour have garnered considerable attention. The aged brain has lower rates of stem cell proliferation [68], less migration of NSPCs, and reduced neurogenesis [69, 70]. Across all neurogenic niches, these changes are concurrent with physical changes to the niche [62]. The ependymal layer thins over time [69], and fewer NSPCs are in contact with the CSF. This leads to a reduction in the signalling molecules that reach NSPCs, and a reduction in their proliferation [70]. Throughout aging, microglia become more densely packed within the niche, and higher proportions of microglia release pro-inflammatory factors (e.g. TNF-α and IL1-β) which decrease NPSC proliferation and attenuate neurogenesis [55, 71]. Astrocytes also contribute to the aging neurogenic niche by reducing Wnt signalling [72], leading to decreased NSPC proliferation [73], impaired neurogenesis, and downregulation of survival genes in NSPCs [72]. Through aging, a number of vascular changes impact NSPC behaviour, including vascular deterioration, increased permeability of blood vessels, and reduced blood flow [74] resulting in a loss of local signalling molecules and regulatory factors that impact proliferation and neurogenesis. Strikingly, when aged animals are exposed to systemic circulating factors from young animals, there is an increase in blood flow, NSPC proliferation, and neurogenesis in the aged CNS [75], restoring NSPC behaviour to that similar in young animals. Conversely, NSPCs derived from the young brain behave like aged NSPCs when exposed to niche factors from the aged brain [73].

Interestingly, it has been shown that prenatal maternal exposure to various factors can lead to profound changes to lifelong neurogenic capacity. Various maternal infections can lead to the activation of pro-inflammatory cytokines present in the neurogenic niche of offspring [76], which then demonstrate dysregulated NSPC expansion and deficits in cognitive function in adulthood [76]. For example, transient maternal infection with IL-6 leads to increased NSPCs in adulthood, aberrant neurogenesis, and perturbations in brain development. These outcomes are attributed to the role of IL-6 in the self-renewal of NSPCs [77]. Thus, niche alterations, whether acting directly or indirectly on NSPCs, can have important and persistent effects on neurogenic capacity.

Injury to the CNS can lead to direct damage to the niche, resulting in a hostile microenvironment for NSPCs. For example, traumatic brain injury (TBI) leads to mechanical injury to the neurogenic niche [78, 79] resulting in edema, gliosis, and dysregulation of neurogenesis [78]. As a result of injury, aberrant migration of neuroblasts can occur. For example, following injury in the hippocampus, migrating cells overshoot their destination in the granule cell layer and become ectopically integrated, leading to aberrant formation of connections with inappropriate targets. Further, changes in intracranial pressure resulting from injury can cause compression of the ventricles and changes to CSF flow [80], impacting NSPC behaviour. Injury-induced damage to endothelial cells within the niche can lead to the release TGF-β [79] which promotes NSC quiescence. Within the spinal cord, similar changes occur to the niche after injury, leading to the release of inflammatory factors which increase NSC proliferation and alter differentiation potential [16]. These examples highlight the importance of considering the niche as a potential therapeutic target, due to its ability to regulate NSPC behaviour.

Neural Stem Cells in Regenerative Medicine

The goal of regenerative medicine is to develop therapeutic strategies to restore tissue following injury and disease. Because of their self-renewal capacity, multipotency, and activation potential, NSPCs present an exciting opportunity to enhance neural regeneration. Neurological disorders can be broadly classified into those in which specific cells are lost over time, termed 'neurodegeneration' and those in which CNS tissue is damaged during an acute event, termed 'acquired injury'. In neurodegeneration, a particular cell type may be affected, for example the targeted loss of oligodendrocytes in Canavan disease [81]. On the other hand, acquired injuries like hypoxia ischemia, TBI, or spinal cord injury can lead to widespread loss of multiple neural cell types.

Considering the plethora of factors that influence NSPCs and their niche, there are numerous opportunities to intervene with therapeutic approaches. These include transplanting exogenous NSPCs, activating endogenous NSPCs, and modifying the microenvironment (for example, by altering inflammation or angiogenesis). These approaches are summarized in the following sections, and in Fig. 3.4.

Exogenous Stem Cell-Based Approaches

One approach that has garnered much interest in the field of neural repair is NSPC transplantation. Using this approach, NSPCs can be expanded and/or fate-directed *in vitro*, and grafted into the neurogenic environment or damaged tissue (Fig. 3.4) [82]. A fundamental challenge to using exogenous stem cells is determining the optimal source of the cells. Early studies focused on using human embryonic stem cells (hESCs), whose pluripotency affords them the ability to generate NSPCs [83]. However, these cells must be obtained from viable embryos, creating ethical concerns that put con-

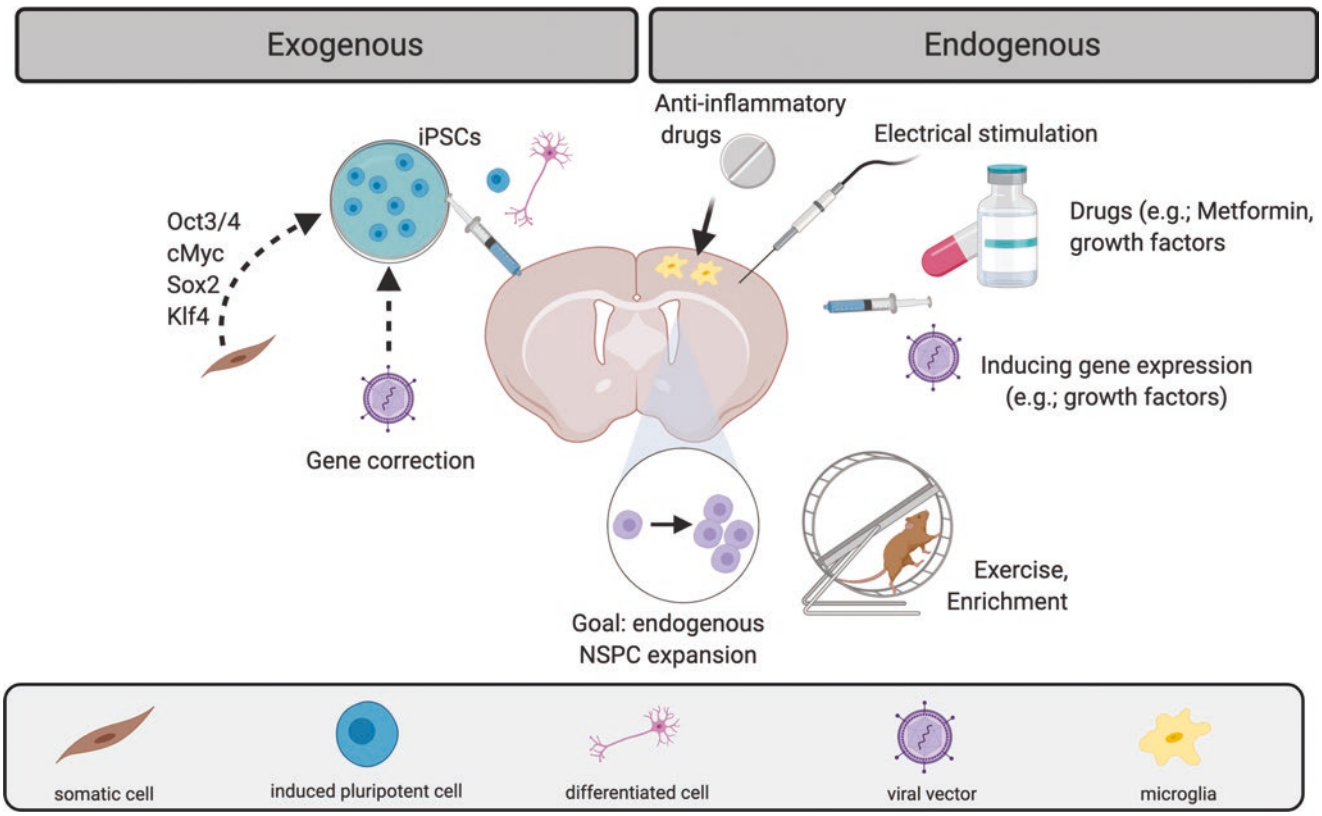


Fig. 3.4 Therapeutic approaches to neural stem cells in regenerative medicine. A plethora of approaches have been explored to use stem cells to repair the central nervous system (CNS) following injury and disease. These can be categorized into exogenous approaches, wherein stem cells are introduced into the CNS, and endogenous, wherein the resident stem cells are targeted for activation. Exogenous approaches (left) include the use of a variety of cell sources such as induced pluripotent stem cells (iPSCs), generated by the addition of Oct 3/4, cMyc, Sox2, and Klf4 to somatic cells [81]. This approach may be complimented by genetic correction or manipulation, and iPSCs may be transplanted in their multipotent state or following differentiation into a more committed cell type. Non-neural stem cell sources, including mesenchymal stem cells, have also demonstrated promise in exogenous therapeutic approaches. Endogenous approaches (right) include the use of electrical stimulation, growth factors, drugs, and/or exercise to stimulate NSPCs; or using anti-inflammatory drugs or induced expression of growth factors or other signalling molecules with the goal of modulating the microenvironment of the injured brain, thereby making it more amenable to endogenous neurogenic processes. *Created with* BioRender.com

siderable limitations on this approach. In 2006, Takahashi and Yamanaka made the seminal discovery that fully differentiated somatic cells could be harvested from a host and induced to a pluripotent state by introducing a combination of four growth factors: Oct3/4, Sox2, c-Myc, and Klf4 [84]. These 'induced pluripotent stem cells' (iPSCs) became the gold standard as a means to produce stem cells for repair throughout the body. While this approach circumvents the ethical issues associated with hSCs and eliminates concerns of immunogenicity, iPSCs have the potential to form teratomas and are not easily controlled once transplanted.

A further limitation of autologously sourced iPSCs is their limited application in the treatment of genetic diseases, as iPSCs contain any genetic mutations present in the host. However, this may be avoided by the application of stem cells in combination with gene therapy technology [85]. Here, autologous stem cells can be obtained from a patient and transduced *ex vivo* with a viral construct containing a corrected or missing gene. These 'modified' multipotent stem cells can then be engrafted back into the patient. This approach has shown promise in the treatment of pediatric degenerative white matter diseases like metachromatic leukodystrophy [86].

Another consideration for exogenous cell-based therapies is whether it is optimal to fate-direct cells prior to transplant. Recent studies have highlighted this approach as both feasible and promising. Glial progenitor cells can be generated from stem cells derived from either human embryonic forebrain or iPSCs; following transplantation, these cells can colonize the recipient brain, migrate throughout the CNS, and remyelinate focal lesions throughout the CNS [87]. This fate-directed approach represents a promising use of exogenous cell transplantation for pediatric neurodegenerative diseases and injuries that affect the white matter.

Endogenous Stem Cell-Based Approaches

An alternative to exogenous stem cell transplantation is the recruitment of endogenous NSPCs [58, 62, 88]. Following CNS injury, NSPCs can be recruited from a quiescent to an activated state, demonstrating a marked increase in proliferation [89]. Many examples of NSPCs migrating from the SEZ niche towards sites of injury have been reported in models of spinal cord injury, neonatal brain injury, and in adult models of injury and disease [58, 88, 90]. However, in mammals, this response is insufficient to support structural and functional repair. Moreover, upon arriving at the injury site, the vast majority of NSPCs do not survive or integrate into existing neural circuitry, but rather generate reactive astrocytes that contribute to the formation of a 'glial scar' [58, 90]. Injury-induced cues that impact migration include factors released from local neurons and glia at the site of injury and chemical cues released from microglia and astrocytes [90, 91] such as SDF1a and matrix metalloproteinases (MMPs) [92, 93]. The hostile microenvironment of the injury site, which includes pro-inflammatory cytokine release from microglia, growth-inhibiting perineuronal nets, extensive cell death, release of neurotoxic factors, and dense physical networks of reactive astrocytes, likely contributes to the poor survival of niche-derived NSPCs and lack of a pro-regenerative response.

Many studies have sought to enhance the endogenous response of NSPCs through stimulation with growth factors, exercise and EE, small molecules, and electrical stimulation (Fig. 3.4). Promising results using epidermal growth factor (EGF) and erythropoetin demonstrated that delivery after stroke increased neurogenesis [94]. However, it remains a challenge to administer growth factors, due to the relatively impermeable blood–brain barrier (BBB), tumorigenic risks associated with mitogen administration [95], and the relatively rapid degradation of therapeutic factors following delivery. Attempts to minimize these challenges include the use of repurposed drugs that can cross the BBB and have an established safety and feasibility profile. Towards this goal, the drug metformin has received much attention. Metformin is a commonly prescribed drug used to treat Type II diabetes that has been shown to have pleiotropic effects in the CNS, including the activation of NSPCs. Metformin has been shown to expand the size of the NSPC pool and promote neurogenesis and oligogenesis [88]. Metformin administration following neonatal and childhood brain injury leads to increased neurogenesis and promotes motor and cognitive recovery [62, 88]. In addition to its effects on NSPCs, metformin reduces inflammation, which likely supports NSPC survival in the injured brain [96]. Moreover, the potential of repurposing an approved therapeutic drug provides the unique potential for fast translation to the clinic [95].

Obstacles, Challenges, and Considerations

Before stem cells can be safely applied therapeutically across a broad range of diseases, there are several significant obstacles to overcome. One fundamental controversy is whether, and for how long, neurogenesis persists in the adult human brain. First demonstrated in the late 1990s [97], the presence of neurogenesis in the post-developmental human CNS has remained under scrutiny as recently as 2018 [98], despite reported neurogenesis in the hippocampus of healthy human subjects across the lifespan [99]. Regardless of the extent of neurogenesis in the adult and aged brain, it is widely accepted that neurogenesis occurs in children and young adults [98]. Nonetheless, harnessing the potential of NSPCs remains a compelling challenge in regenerative medicine.

Despite a vast body of research demonstrating the beneficial effects of stem cell-based regenerative therapies outlined in this chapter, some approaches remain far from being safely translated into humans. Preclinical studies rely on the use of animal models to study neurorepair, with varying degrees of validity. Most injury and disease models are induced chemically or pharmacologically, or via genetic manipulations that may present similar neuropathology to the clinical condition while having a different genetic basis. Further, studies are often performed in young and otherwise healthy animals, not accounting for the confounding effects of age and comorbidities. Taken together, these studies frequently lack the heterogeneity of the clinical population, resulting in the failure of many therapies to show benefit in clinical conditions.

The CNS also presents unique challenges in terms of gaining access with therapeutics. When considering the application of regenerative strategies based on NSPC recruitment, finding factors that can pass the BBB remains difficult. Under physiological conditions, the BBB allows for the precise control of homeostasis and protection from pathogens and toxins from entering the CNS. However, it serves as a double-edged sword in the context of the injured and diseased CNS, acting as a major obstacle for effective delivery of therapeutic compounds. Although injury and disease can lead to disruption of the BBB, changes in permeability are dynamic over the first hours or days. However, the BBB may be bypassed, for example, by nasal administration, wherein unique connections between the olfactory and trigeminal nerves provide direct access to the brain, or bioengineering materials may be used that allow direct, local, and sustained delivery of factors that regulate NSPC behaviour directly in the damaged region [100]. Such approaches are of considerable therapeutic interest.

Summary and Conclusions

The discovery of NSPCs in the post-developmental CNS has led to an abundance of research into their role in development and beyond. NSPCs are responsible for the development of the CNS from a neuroepithelial layer in embryogenesis and persist in the CNS in defined neurogenic niches where they remain capable of generating neural cells throughout life. To date, the complexity, heterogeneity, and potential of NSPCs continue to be revealed, as evolving technologies allow for dramatically increased resolution of the inner workings of these cells. Current studies aimed at identifying and assessing the potential of distinct NSC populations continue to drive a more intricate understanding of the diversity of NSCs and help target specific subpopulations for neurorepair (e.g. pNSCs). The CNS is complex, containing numerous specialized cell types and complex circuitry, which renders it particularly vulnerable to injury. As such, exploring NSC heterogeneity is crucial to improve our knowledge of fundamental NSC biology and exploit the potential of these cells. While the mammalian CNS exhibits limited recovery following injury and disease, NSPCs hold enormous potential for promoting neuroplasticity and repair. To date, attempts to harness this potential have proven challenging and further research is warranted. Approaches that target both the cells and their niche will likely prove most powerful in the application of NSPCs for regenerative medicine. Although the original author of the belief that the CNS is irrevocably immutable, Roman y Cajal himself added, '*it is for the science of the future to change, if possible, this harsh decree*' [1]. With increasing technology, evidence suggests that we are now unlocking the potential of NSPCs to do just that.

Multiple Choice Questions (Answers below)

Q1. Which of the following cells have the properties of neural stem cells in the developing brain?

i. Radial glia
ii. Ependymal cells
iii. Primitive neural stem cells
iv. Definitive neural stem cells
v. Induced pluripotent stem cells

A. All of the above
B. None of the above
C. Only i, ii, iii and iv
D. Only ii, iii and iv
E. Only i, iii, and iv

Q2. Which of the following is NOT a marker of primitive neural stem cells?

A. LIF receptor
B. Oct4
C. c-Kit receptor
D. GFAP
E. Nestin

Q3. Choose the **most correct** statement:

A. Neural stem cells are found in rare numbers through the adult brain parenchyma
B. Microglia are derived from radial glia during development
C. Neuroblasts are found in abundance in the periventricular region of the adult spinal cord
D. Vascular endothelial cells comprise an important part of the neural stem cell niche
E. All of the above are true

Answers

1. (E)
2. (D)
3. (D)

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Neurotrophins and Cell Death

4

Arman Shekari, Crystal Mahadeo, Nisha Sanwalka, and Margaret Fahnestock

Abbreviations

Akt	Protein kinase B
APAF-1	Apoptotic protease activating factor 1
ASD	Autism spectrum disorder
BDNF	Brain-derived neurotrophic factor
BMP	Bone morphogenic protein
Cl^-	Chloride
CNS	Central nervous system
CREB	cAMP response element-binding protein
Cyt c	Cytochrome c/ cytochrome complex
DLPFC	Dorsolateral prefrontal cortex
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglia
E	Embryonic day
Elk-1	E26 transformation-specific-like protein 1
E-LTP	Early-phase long-term potentiation
ENS	Enteric nervous system
EPS-8	Epidermal growth factor receptor kinase substrate 8
ERK	Extracellular signal-regulated kinase
FADD	Fas-associated protein with death domain
FRS2	Fibroblast growth factor substrate 2
GABA	ɣ-aminobutyric acid
GDI	Guanine-nucleotide dissociation inhibitor
GDNF	Glial cell line-derived neurotrophic factor
GRB2	Growth factor receptor-bound protein 2
IPSC	Inhibitory postsynaptic potential
JNK	c-Jun N-terminal kinase
KCC2	Potassium chloride cotransporter 2
LGN	Lateral geniculate nucleus
LTD	Long-term depression
LTP	Long-term potentiation
L-LTP	Late-phase long-term potentiation
MAG	Myelin-associated glycoprotein
MAP	Mitogen-activated protein kinase
MBP	Myelin basic protein
mEPSC	Mini excitatory postsynaptic current
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
mTOR	Mammalian target of rapamycin
NKCC1	Na-K-Cl cotransporter
NMDA	N-methyl-D-aspartate receptor
NGF	Nerve growth factor
NF-κB	Nuclear factor-κB
Nogo-R	Nogo-66 receptor
NR2B	N-methyl D-aspartate receptor subtype 2B
NRAGE	Neurotrophin receptor-interacting melanoma antigen gene homolog
NRIF	Neurotrophin receptor interacting factor
NT-3	Neurotrophin 3
NT-4	Neurotrophin 4
OMgp	Oligodendrocyte-myelin glycoprotein
P	Postnatal day
$p75^{NTR}$	Pan-neurotrophin receptor
PCD	Programmed cell death
PI3K	Phosphoinositide-3′-kinase
PKC	Protein kinase C
PLC-γ1	Phospholipase C gamma-1
PNS	Peripheral nervous system
PSD95	Postsynaptic density protein 95
SC-1	Schwann cell factor 1
SHC	Src homology 2 domain containing
SNP	Single-nucleotide polymorphisms
SorCS2	Sortilin-related VPS10 domain containing receptor 2
SOS	Son of sevenless
RhoA	Ras homolog family member A
ROCK	Rho-associated protein kinase
TGF-β	Transforming growth factor-β
TLE	Temporal lobe epilepsy
TRAF	Tumor necrosis factor receptor-associated factor
Trk	Tropomyosin-related kinase

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Learning Objectives

1. Understand why cell death is important in neural development
2. Understand the biochemical mechanisms underlying apoptosis
3. Identify the different neurotrophin receptors, which neurotrophins they bind and describe their function in nervous system development
4. Understand and identify neurotrophin signaling pathways and their corresponding biological effects
5. Understand how abnormalities in neurotrophic signaling can lead to developmental disorders

Highlights

- Brain development progresses as a balance between early cell proliferation and abundance and pruning of connections and synapses over time.
- Neurotrophins are a group of four: 1. Nerve growth factor (NGF), 2. Brain-derived neurotrophic factor (BDNF), 3. Neurotrophin 3 (NT-3), and 4. Neurotrophin 4 (NT-4); each of which exerts trophic effects on different brain cell subpopulations.
- Dysregulation of the neurotrophins has been found to result in a host of developmental disorders including autism, epilepsy, and fetal alcohol spectrum disorder.

Developmental Cell Death and Brain Development

Overproduction of the number of neurons and the connections between them occur initially during nervous system development. Throughout development, this initial surplus of cells is reduced and refined through a process termed programmed cell death (PCD). PCD has been known about since the mid-nineteenth century in the context of amphibian metamorphosis and has been studied in the context of development for over 60 years [1]. Classically, PCD serves three functions during development: (1) phylogenetic regulation, the removal of unneeded structures; (2) morphogenic regulation, the shaping of required structures; and (3) histogenic regulation, the reduction of cell numbers. PCD is an evolutionarily conserved process that occurs in both vertebrates and invertebrates. PCD is also very tightly controlled, as evidenced by the exactly 131 cells that undergo PCD during normal development in *Caenorhabditis elegans*, 105 of these cells being of neuronal origin [2].

The biochemical processes that underlie cell death include autophagy and apoptosis. Autophagy is often described as a process of cellular "self-eating" where the cell digests its subcellular structures in a controlled fashion [1]. This process is carried out by the autophagosome, a spherical membranous sac that engulfs other organelles and delivers them to the lysosome for eventual breakdown. Classically, autophagy has been described as occurring principally in response to various cellular stressors including infection, starvation, or acute injury. As a result, developmental research has largely focused on apoptosis, described as controlled cell death, biasing the literature toward exploring the importance of apoptotic pathways during development. While this chapter will focus on apoptosis, as it is the predominant form of cell death in the developing brain, it is important to appreciate that cell death during development is not homogenous in terms of its governing biological processes, as knockout of autophagy-related genes has significant developmental consequences.

Apoptosis is a tightly controlled form of cell death that occurs in response to external factors including the binding of specific "death ligands" to membrane receptors and internal factors such as DNA damage [3]. Regardless of the nature of the signal, once it is received, the cell begins to cleave many of its subcellular structures using enzymes called caspases. The production and activation of caspases are carried out by the cell itself. In other words, cells have endogenous programming that causes them to cease functioning if they encounter an apoptotic signal. Normal development involves the employment of these signals in a controlled fashion to reduce and refine the neuronal population.

Caspases are initially translated as zymogens, inactive precursor enzymes that are activated by proteolytic cleavage [4]. Multiple mechanisms for caspase activation exist in the cell. The canonical pathway (Fig. 4.1) involves the binding of the Fas ligand to the extracellular domain of the Fas receptor, leading to the intracellular recruitment of the Fas-associated protein with death domain (FADD) adaptor protein, among others, which in turn leads to the recruitment of procaspase-8 to the bound receptor. Procaspase-8 is converted to active caspase-8 through autolytic cleavage via association with the activated receptor complex. Caspase-8 then activates the other latent procaspases in the cell [4, 5]. Caspase activation is further stimulated by the release of cytochrome c from mitochondria during apoptosis. Cytochrome c release can initiate this apoptotic cascade in the event of a breach of the outer mitochondrial membrane, an event that often occurs in damaged or unhealthy cells [5]. Cytochrome c binds monomeric apoptotic protease activating factor 1 (APAF-1) in the cytosol, leading to its dimerization and activation of procaspase-9 and subsequent downstream caspases [5, 6].

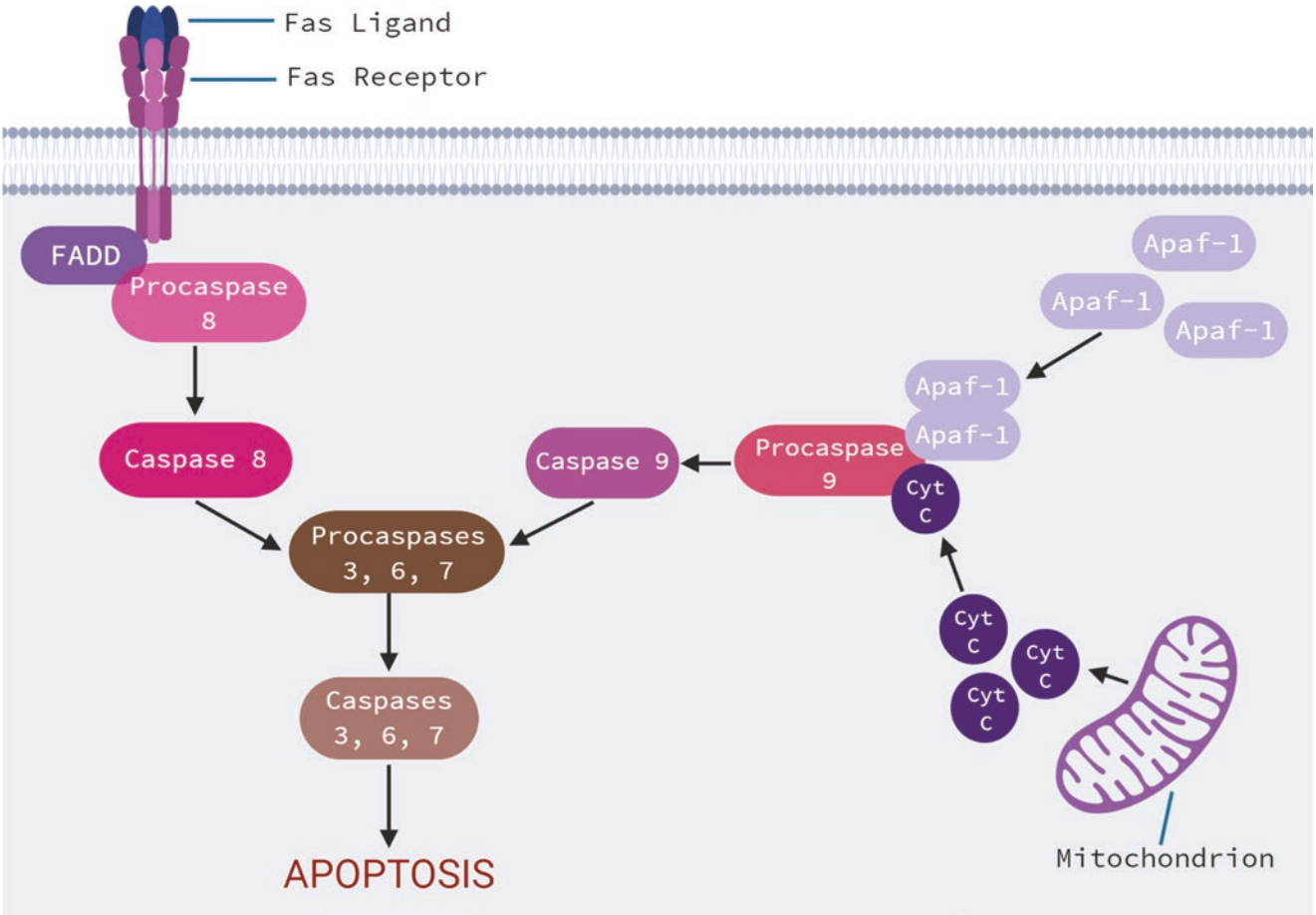

Fig. 4.1 The canonical caspase activation pathway. Extracellular cues, including the binding of the Fas ligand to its receptor, lead to the recruitment of the Fas-associated protein with death domain (FADD) adaptor protein. FADD protein binding leads to the recruitment of procaspase-8 to the bound receptor. Procaspase-8 is converted to active caspase-8 through autolytic cleavage via association with the activated receptor complex. Caspase-8 then activates the other latent procaspases in the cell that carry out apoptosis by cleaving subcellular structures. Apoptotic signaling can be either initiated or further stimulated by intracellular cues including the release of cytochrome c (CytC) from damaged mitochondria. CytC binds monomeric apoptotic protease activating factor 1 (APAF-1) in the cytosol, leading to its dimerization, cleavage of procaspase-9 to caspase-9 and subsequent activation of downstream caspases

A straightforward example of the importance of this controlled apoptosis is illustrated by two diseases involving abnormal brain volume, macrocephaly (enlarged head) and microcephaly (small head). While other factors aside from abnormal PCD contribute to these disorders, dysfunction of caspase signaling pathways leading to too much or too little apoptosis have been shown to cause drastic volumetric abnormalities in the brain. For example, overactivation of the caspase-activating c-Jun-amino-terminal kinase (JNK) pathway results in microcephaly of forebrain structures in mice due to increased caspase activity [7]. Furthermore, the regulation of caspase activity in the brain is region-specific and affects the structure of the brain independent of its volume by influencing other apoptosis-contingent processes like neural tube closure [7, 8].

Due to its importance in neurodevelopment, apoptosis must be tightly regulated. Seminal work done by Hamburger and Levi-Montalcini in the mid-twentieth century demonstrated that the size of peripheral ganglia in the developing nervous system is contingent on growth factors secreted by target tissue, e.g., tissue innervated by those ganglia [9, 10]. Neurons in the developing nervous system compete with their counterparts to establish connections with their respective targets, which in turn reinforce these connections by secreting growth factors that sustain the connecting cell. The supply of these growth factors is limited by the target tissue itself; neurons that do not make strong connections with their respective targets receive insufficient amounts of growth factor and subsequently undergo apoptosis. Further work by Levi-Montalcini led to the discovery of nerve growth factor (NGF), the substance responsible for target-derived apoptotic control in the periphery [10]. NGF belongs to a class of proteins known as neurotrophic factors, secreted proteins that act in the

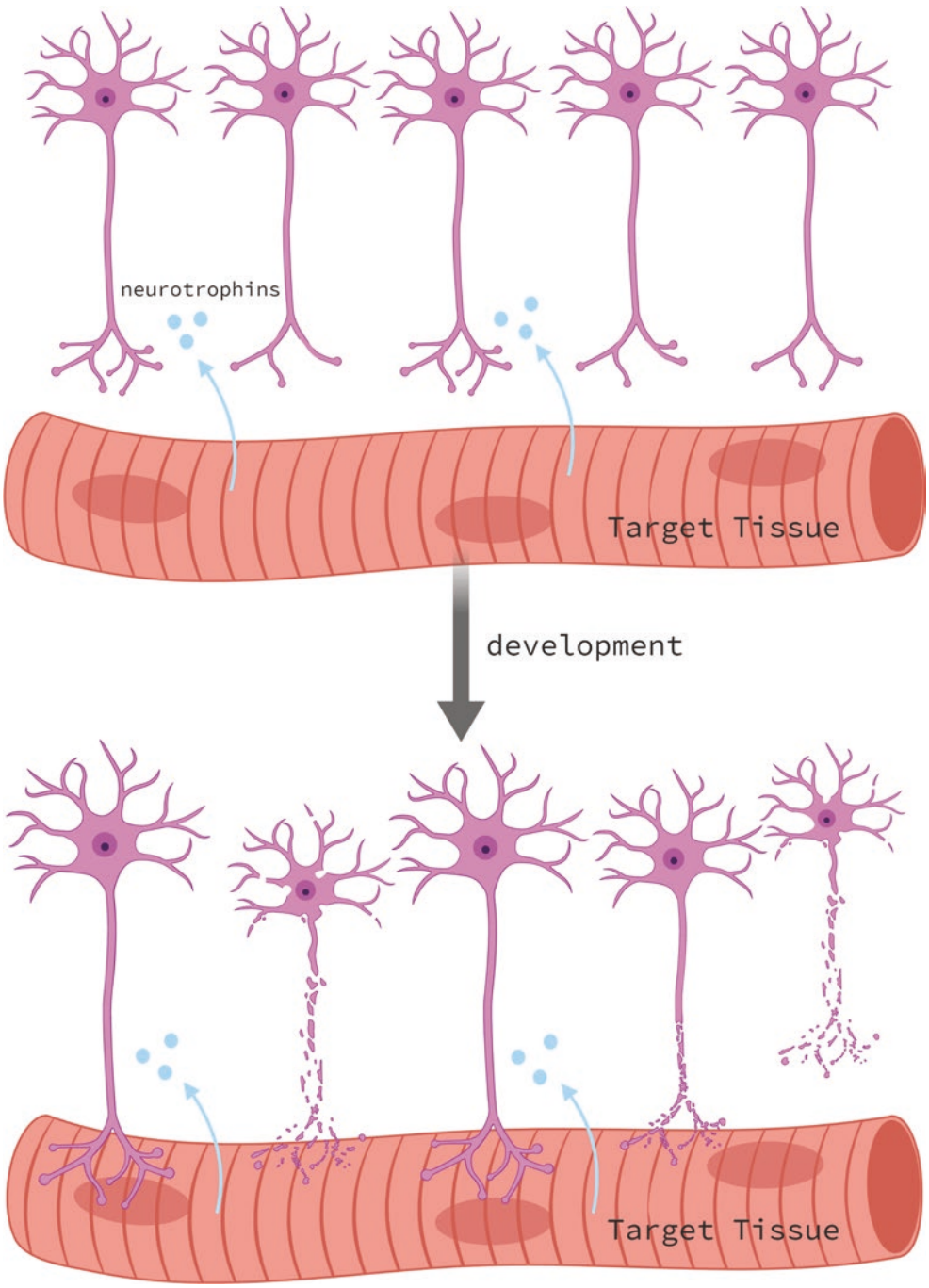

Fig. 4.2 The neurotrophic theory of development. Elimination of surplus neurons during development is driven by competition for target-secreted neurotrophic factors. Neurons that do not make enough robust connections with their targets receive insufficient trophic support and are eliminated via apoptosis

nervous system to regulate PCD during development, along with an entire host of other important functions that extend into postnatal life.

Subsequent research resulted in the formation of the "trophic theory of development." The trophic theory expands on the work of Levi-Montalcini and describes how target-derived neurotrophic factors regulate apoptosis during development. The theory states that neuronal elimination during development is driven by competition for finite amounts of trophic factors secreted by target tissue [10] (Fig. 4.2). The following sections will discuss in-depth neurotrophic factors, their signaling, and how they regulate apoptosis during neurodevelopment.

Neurotrophins

Neurotrophic factors are members of the growth factor family of proteins that typically act on central and peripheral neurons during development and throughout life.

Neurotrophic factors are signaling proteins that play a critical role in neural development, differentiation, axonal growth, target innervation, migration, plasticity, and function [11]. During development, neurotrophic factors secreted by target cells provide trophic support for axonal growth and axonal projections from innervating neurons that have not yet reached their final targets [12, 13]. While some neurons generate their own (autocrine) neurotrophic factors, neurotrophic factors can also be obtained from neighboring or distant (paracrine or target-derived) neuronal and non-neuronal cells [14].

Neurotrophins are a family of neurotrophic factors that are similar in structure, all dimeric in nature and stabilized by a cysteine knot. There are four known neurotrophins found in mammals: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4), all of which exert trophic effects on different and sometimes overlapping neuronal subpopulations and demonstrate unique expression patterns in the central nervous system (CNS) and the peripheral nervous system (PNS) [11]. More than any other family of neurotrophic factors, neurotrophins have been extensively studied for their roles in development. Therefore, the remainder of this chapter will focus on the neurotrophins, with the understanding that other neurotrophic factors, such as the glial cell line-derived neurotrophic factor (GDNF) family, act in similar ways upon different subsets of neurons.

NGF was the first neurotrophin to be characterized. It was discovered by Rita Levi-Montalcini, Viktor Hamburger, and Stanley Cohen in 1956. This finding earned Rita Levi-Montalcini and Stanley Cohen the 1986 Nobel Prize in Physiology or Medicine [15]. NGF is most known for its key role in the development and organization of the nervous system and for its regulation of apoptosis. Almost 30 years later, BDNF was purified from pig brain by Yves-Alain Barde and Hans Thoenen [16]. BDNF is most widely known for its effects on neuronal excitability and synaptic plasticity. NT-3 [17, 18] and NT-4 (also known as NT-5 or NT-4/5) [19–21] were later described by researchers in the 1990s. NT-3 has been extensively studied for its role in nervous system development, while NT-4 has been less studied, possibly because of its low expression levels compared to the other neurotrophins [11].

Neurotrophins and their Receptors

Neurotrophins bind to tropomyosin-related kinase (Trk) receptors, single-pass transmembrane receptor tyrosine kinases that regulate cell survival, growth, differentiation, and apoptosis in the central and peripheral nervous systems (Fig. 4.3). There are three known Trk receptors in mammals: TrkA (encoded by the *NTRK1* gene), TrkB (*NTRK2* gene), and TrkC (*NTRK3* gene) [22]. Trks are similar in structure, with all receptors containing an extracellular ligand-binding domain that consists of two immunoglobulin-like regions with unique ligand-binding specificity, a leucine-rich motif, a single-pass transmembrane domain, a cytoplasmic domain with tyrosine kinase activity, and a 15-amino acid carboxyl-terminal tail. Upon binding of their dimeric neurotrophin ligands, Trk receptors dimerize and autophosphorylate their cytoplasmic tyrosine kinase domains to become activated [22].

Preferential binding to individual Trk receptors is exhibited by all neurotrophins. NGF binds preferentially to TrkA, while BDNF and NT-4 bind TrkB, and NT-3 binds TrkC. NT-3 also binds to the other Trk receptors (A and B) with lower affinity [22]. It should be noted that differential splicing of Trk mRNAs results in multiple isoforms for all Trk receptors, resulting in differences in expression, binding specificity, and function. The TrkA receptor has three isoforms: TrkA-I, TrkA-II, which lack exon 9 in the extracellular domain and TrkA-III which lacks exons 6, 7, and 9 [22]. TrkA-I is found in neuronal cells, whereas TrkA-II is highly expressed in nonneuronal cells and exhibits increased activation by NT-3. TrkA-III is an early developmental form that is constitutively active, independent of ligand. TrkA-III expression is limited to neural crest cells and neural progenitor cells [22]. TrkB has four isoforms, two that contain the tyrosine kinase domain and are referred to as full-length TrkB (TrkB-FL), and two truncated TrkB isoforms (TrkB-SHC and TrkB-T1) that lack parts of the kinase domain with or without the SHC-binding site, respectively, and therefore cannot signal as does TrkB-FL. Truncated TrkB acts primarily as a dominant-negative receptor that inhibits the activity of TrkB-FL when bound to it. The two truncated TrkB isoforms are generally found on glial cells and at extrasynaptic sites and may also act as inhibitors of neuronal TrkB-FL activity by sequestering BDNF [23]. However, there is recent evidence suggesting that truncated TrkB isoforms can signal on their own by releasing intracellular calcium stores in response to BDNF binding [24]. The TrkC receptor has both full-length (2) and truncated (4 known) isoforms similar to those of TrkB, with the full-length isoforms having tyrosine kinase activity and the truncated forms (TrkC-T1 being predominant) lacking the tyrosine kinase domain and acting as TrkC-FL inhibitors [22].

The pan-neurotrophin receptor ($p75^{NTR}$) binds all pro and mature neurotrophins (Fig. 4.3). $p75^{NTR}$ is a single-pass transmembrane protein and a member of the tumor necrosis factor receptor (TNFR) superfamily of proteins [25]. $p75^{NTR}$ is a non-catalytic receptor that consists of a glycosylated extracellular ligand-binding region that has four cysteine-rich regions, a single transmembrane segment, and a cytoplasmic sequence known as the death domain. A string of basic amino acids common to all neurotrophins comprises

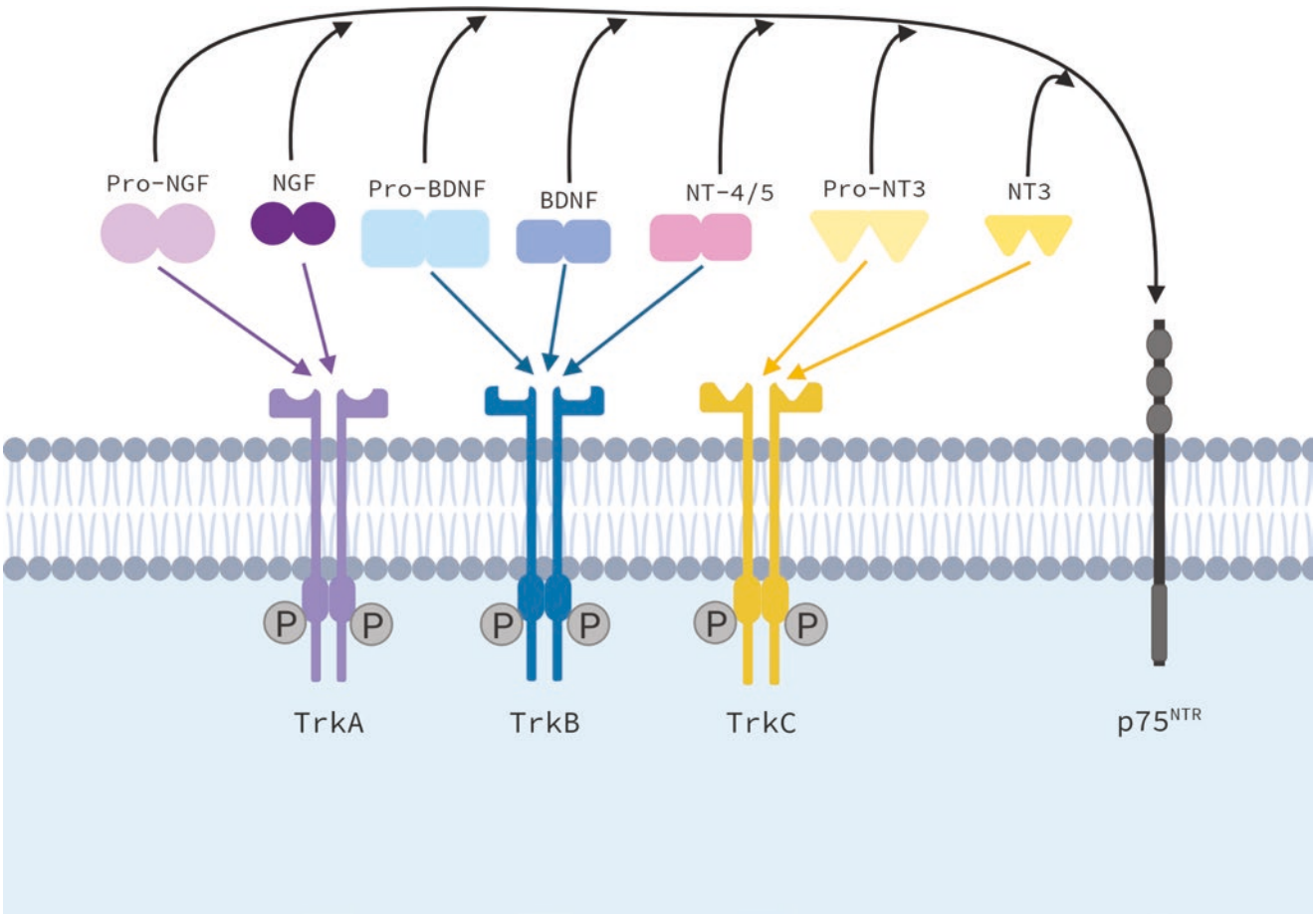

Fig. 4.3 The binding of neurotrophic factors to their respective receptors. All pro and mature neurotrophins bind to one or more Trks and to $p75^{NTR}$

the binding domain to the negatively charged $p75^{NTR}$. Neurotrophin binding to this receptor results in the recruitment of adapter proteins to its cytoplasmic death domain, activating several different signaling pathways that are important for the balance of neuronal survival and death throughout development. The recruitment of individual adapter proteins and their different downstream signaling pathways are dependent upon both the ligand and the transmembrane binding partners of $p75^{NTR}$ [25].

During development, $p75^{NTR}$ is involved in the regulation of survival and neurite outgrowth [25]. Binding of neurotrophins to $p75^{NTR}$ results in the activation of pro-apoptotic or pro-survival signaling cascades and neurite outgrowth or neurite retraction cascades, depending on the co-receptor that is recruited (Fig. 4.4). In cells that express both $p75^{NTR}$ and Trk receptors, these two receptors work together to promote survival, whereas in cells that lack neurotrophic support, $p75^{NTR}$ signals cell death. $p75^{NTR}$ can interact with all Trk receptors at both the extracellular and intracellular domains resulting in conformational changes that alter their function. The interaction between Trk receptors and $p75^{NTR}$ can enhance the affinity of Trk receptors for individual neurotrophins [26]. For example, high-affinity NGF binding to TrkA is contingent upon the presence of $p75^{NTR}$ [27]. The association of TrkA with $p75^{NTR}$ leads to recruitment of TrkA to lipid rafts, increased signaling through TrkA-mediated neurotrophic pathways and inhibition of $p75^{NTR}$ apoptotic pathways [25]. Neurite outgrowth is also regulated by $p75^{NTR}$ signaling. Specifically, neurotrophin binding to Trks leads to the activation of Ras, causing neurite outgrowth, whereas $p75^{NTR}$ signaling leads to activation of Rho and neurite outgrowth inhibition.

While the $p75^{NTR}$ receptor can signal in isolation, it can also act as a co-receptor and form complexes with other receptors in addition to Trks, altering its signaling (Fig. 4.4). $p75^{NTR}$ associates with the Nogo-66 receptor (Nogo-R) in the presence of Lingo-1 to form a ternary complex that inhibits neurite outgrowth and axonal regeneration in injured neurons when bound by one of its ligands (Nogo, myelin-associated glycoprotein [MAG] or oligodendrocyte-myelin glycoprotein [OMgp]) [28]. Additionally, $p75^{NTR}$ can form a ternary complex with Sortilin-related VPS10 domain containing receptor 2 (SorCS2) and Trio; binding of proNGF to $p75^{NTR}$ and release of Trio results in growth cone collapse.

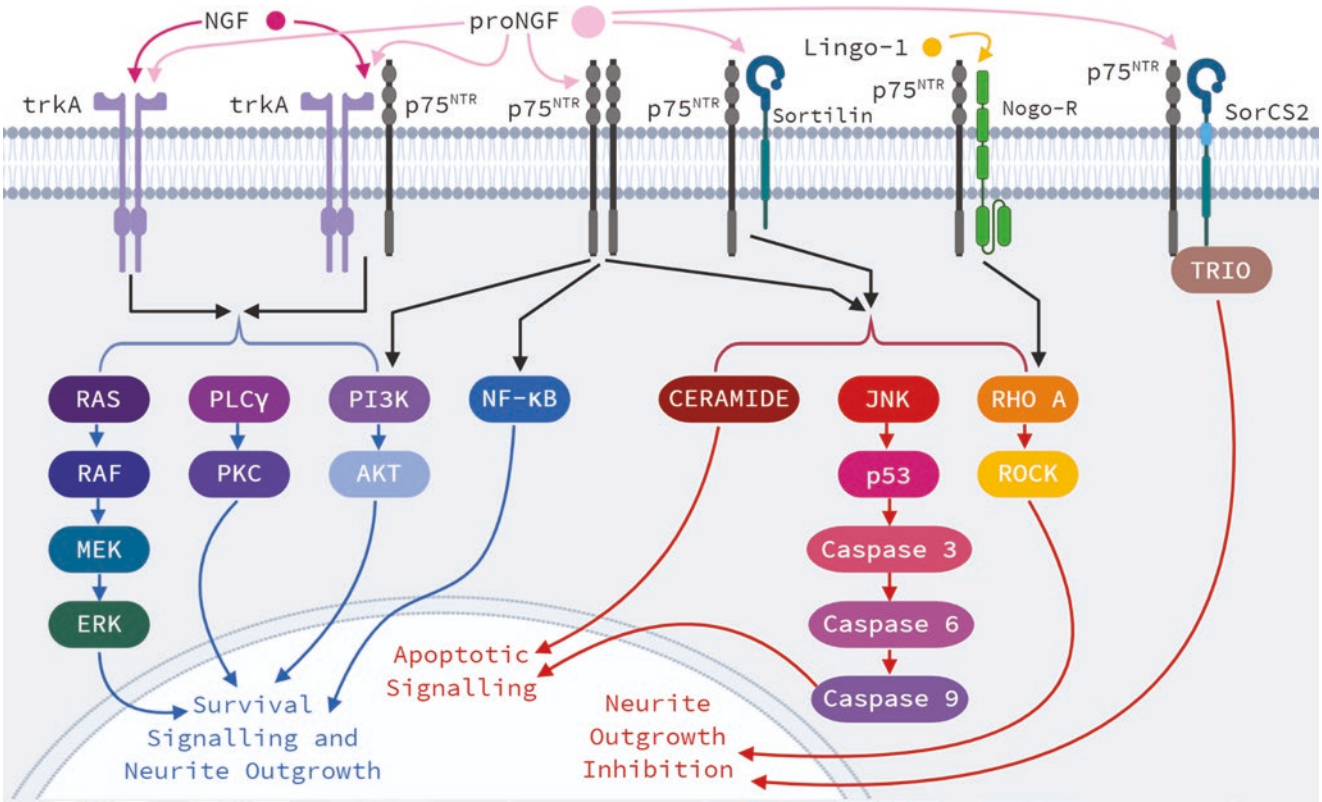

Fig. 4.4 The various pathways activated by TrkA and p75^{NTR}. TrkA dimers in isolation activate pro-survival pathways including the Ras-MAP kinase, PLC-γ, and PI3 kinase/Akt pathways. p75^{NTR}, when complexed with TrkA, increases pro-survival signaling through TrkA. p75^{NTR} in isolation can also signal survival via the NF-κB and PI3 kinase/Akt pathways. p75^{NTR} in isolation activates apoptosis via ceramide, JNK, c-Jun phosphorylation and caspases 3, 6, and 9. When complexed with sortilin, p75^{NTR} binds proNGF with higher affinity than NGF, signaling cell death. p75^{NTR} can also cause growth cone retraction/neurite outgrowth inhibition when complexed with the Nogo-66 receptor (Nogo-R) and its ligand Lingo-1. Additionally, p75^{NTR} can complex with Sortilin-related VPS10 domain-containing receptor 2 (SorCS2) and Trio; binding of proNGF to p75^{NTR} triggers the release of Trio resulting in growth cone collapse

Importantly, the association of sortilin and p75^{NTR} in the presence of proNGF signals apoptosis [29]. Ultimately, it is the balance of these receptors in any given cell that determines the signaling pathway activated and its functional consequences [25, 30, 31].

Downstream Signaling

Neurotrophin receptor activation can have cell survival or apoptosis, neurite growth, or retraction outcomes, depending on the combination of receptors and the signal transduction pathways activated. Binding of neurotrophins to Trk receptors results in dimerization of the receptor, triggering its autophosphorylation at multiple intracellular amino acid residues. This leads to the recruitment of adaptor proteins such as Src homology 2 domain containing (SHC), growth factor receptor-bound protein 2 (GRB2), and son of sevenless (SOS) and subsequent activation of intracellular target proteins (Fig. 4.5). These include phospholipase C gamma-1 (PLC-γ1) and p85, which release calcium from intracellular stores and activate protein kinase C (PKC), and the Ras-mitogen activated protein (MAP) kinase pathway; initiation of the phosphoinositide-3′-kinase (PI3K)-Protein kinase B (Akt) signaling pathway which promotes neuronal survival, dendritic growth, and spine maturation; and recruitment of fibroblast growth factor receptor substrate 2 (FRS2) to the adaptor protein complex containing SHC, GRB2, and SOS, resulting in activation of the Ras-MAP kinase pathway, promoting neurite outgrowth, and neuronal differentiation [11, 32].

Neurotrophins binding to p75^{NTR} results in the recruitment of adapter proteins such as neurotrophin receptor-interacting melanoma antigen gene homolog (NRAGE), tumor necrosis factor receptor-associated factors (TRAFs), neurotrophin receptor-interacting factor (NRIF), Schwann cell factor 1 (SC-1), and Ras homolog family member A (RhoA) to the p75^{NTR} intracellular domain. These activate several signal transduction pathways. TRAFs activate nuclear factor-κB (NF-κB), which induces either programmed cell death or survival, depending on the cell type and the presence or absence of Trks. NRIF activates the Jun

N-terminal kinase (JNK) pathway, which induces programmed cell death through the activation of tumor protein p53 resulting in downstream caspase activation. Sphingomyelinase activation produces ceramide, a pro-apoptotic or pro-mitogenic substance in different cell types. Additional adapter proteins can activate other signaling cascades such as NRAGE and SC-1 which activate pathways that lead to exit from the cell cycle and growth arrest or to regulation of neurite outgrowth via the RhoA pathway [11, 25] (Fig. 4.5).

Binding of myelin-associated growth inhibitors to the Nogo-R-Lingo-1-p75^{NTR} complex activates RhoA to inhibit axon growth. ProNGF binding to a complex of sortilin and p75^{NTR} activates a pro-apoptotic signaling cascade through the JNK pathway [29]. Sortilin appears to enhance the pro-apoptotic effects of p75^{NTR} and in some cases is necessary for pro-apoptotic signaling. Taken together, through binding different co-receptors, p75^{NTR} can elicit a myriad of effects through different signaling pathways [25].

Most of these signaling pathways culminate in the activation of transcription factors in the nucleus that result in altered gene expression and the transcription of proteins that carry out distinct functions. For example, BDNF binding to TrkB activates the Ras-MAP kinase pathway that activates

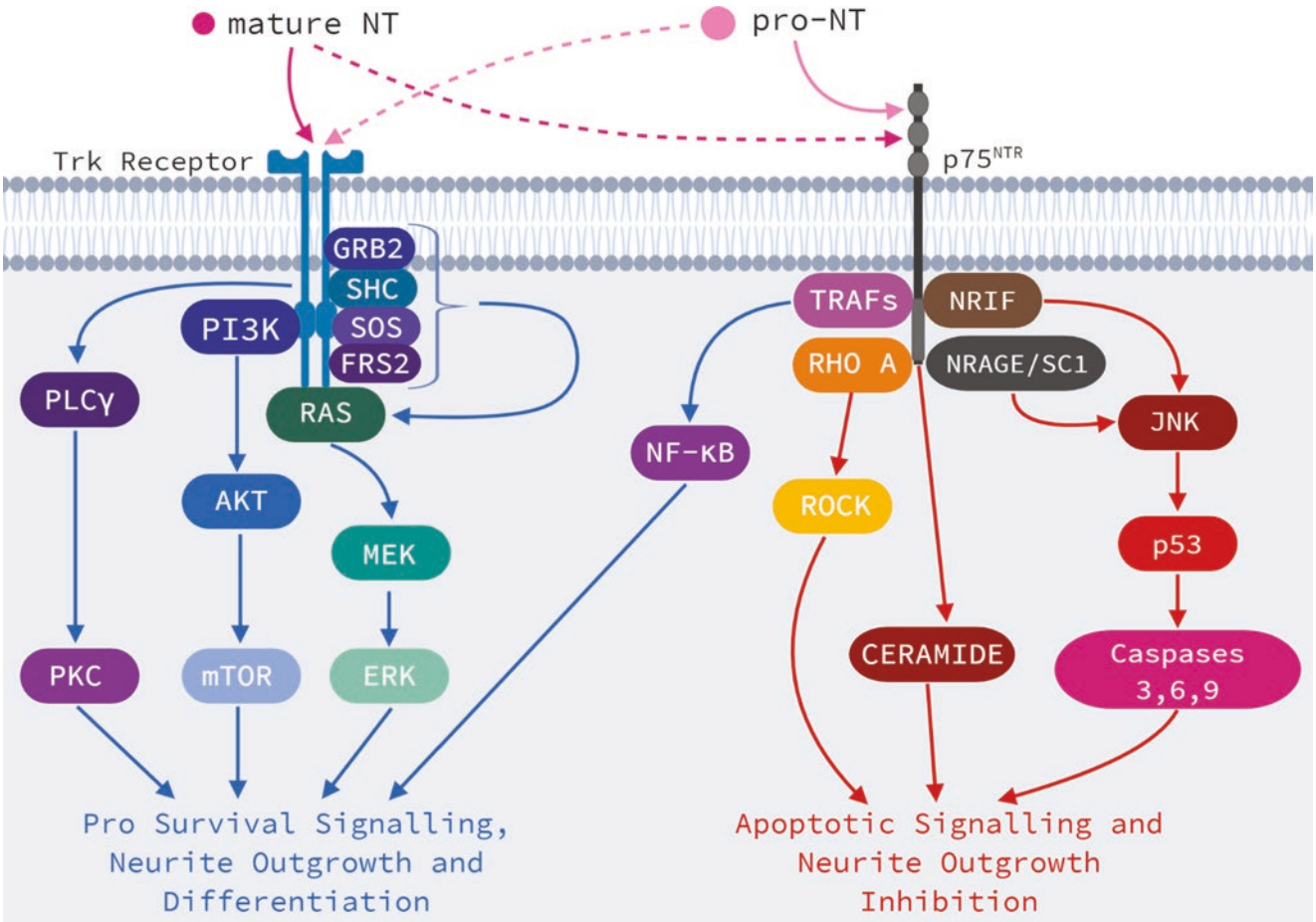

Fig. 4.5 Pathways activated by neurotrophins binding to Trk receptors or p75^{NTR}. Mature neurotrophins bind more strongly to Trk receptors, while proneurotrophins bind more strongly to p75^{NTR}. Binding of either pro or mature neurotrophins to Trk receptors results in the recruitment of adaptor proteins such as Src homology 2 domain containing (SHC), growth factor receptor-bound protein 2 (GRB2) and son of sevenless (SOS). The recruitment of intracellular adapters activates intracellular target proteins phospholipase C gamma-1 (PLC-γ1) and p85, which release calcium from intracellular stores and activate protein kinases. These include protein kinase C (PKC) and Ras-mitogen-activated protein (MAP) kinase, among others. Activation of these kinases leads to the initiation of the phosphoinositide-3′-kinase (PI3K)-Protein kinase B (Akt) signaling pathway, which promotes neuronal survival, dendritic growth, and spine maturation. The binding of either pro or mature neurotrophins to p75^{NTR} triggers the recruitment of adapter proteins such as neurotrophin receptor-interacting melanoma antigen gene homolog (NRAGE), Schwann cell factor 1 (SC-1), tumor necrosis factor receptor-associated factors (TRAFs), neurotrophin receptor-interacting factor (NRIF), and Ras homolog family member A (RhoA) to the p75^{NTR} intracellular domain. TRAFs activate nuclear factor-κB (NF-κB), which induces either programmed cell death or survival, depending on the cell type and the presence or absence of Trks (see Fig. 4.4). NRIF activates the c-Jun N-terminal kinase (JNK) pathway, which induces programmed cell death through the activation of tumor protein p53 resulting in downstream caspase activation

the transcription factor cAMP response element-binding protein (CREB) [32]. CREB is implicated in neurotrophin-induced transcriptional regulation governing protein expression contributing to neurite outgrowth and cell survival. Binding to $p75^{NTR}$, on the other hand, can activate JNK, leading to phosphorylation of the c-Jun transcription factor governing transcription of apoptotic genes. Many other transcription factors and cofactors are activated by neurotrophin signaling pathways as well, resulting in the transcription of pro-survival or pro-apoptotic genes [25].

Pro-Neurotrophins

Like many secreted proteins, neurotrophins are initially translated as proproteins that can be proteolytically processed by enzymes to their mature forms. proNGF and proBDNF can be cleaved intracellularly by furin and extracellularly by plasmin and matrix metalloproteinases [33]. It was initially believed that proneurotrophins were unable to signal independently of their mature isoforms and that their existence served merely to aid protein folding during synthesis. However, it is now understood that proneurotrophins are not necessarily cleaved and can signal independently from their mature counterparts.

Mature BDNF and proBDNF are both found in the brain (Figure 4.6a). While BDNF binds preferentially to TrkB and has less affinity for $p75^{NTR}$, proBDNF binds preferentially to $p75^{NTR}$, making proBDNF a positive apoptotic regulator [34]. The impact of proBDNF on neurons is antithetical to that of BDNF. While BDNF binding to TrkB promotes differentiation, long-term potentiation, spine maturation, and synaptic strengthening, proBDNF binding to $p75^{NTR}$ promotes long-term depression, neurite retraction, synaptic weakening, and apoptosis [35]. As a result, the balance between mature and proBDNF and their receptors is crucial for proper synaptic function and plasticity. Imbalances between mature and proBDNF levels and their receptors have been implicated in neurodevelopmental diseases like autism spectrum disorder and epilepsy, which are discussed later in this chapter.

While proBDNF exhibits opposing effects compared to its mature counterpart, the same cannot be said for proNGF. Initially, proNGF was thought to be a purely apoptotic protein because of its ability to bind and activate $p75^{NTR}$. However, proNGF was also shown to exhibit neurotrophic activity by binding to TrkA. ProNGF is the only form of NGF in both the human and rodent brain as well as in many tissues in the periphery, with little to no mature NGF being present [36] (Figure 4.6b). The activity of proNGF is contingent upon the receptor complement of the cell: in the presence of TrkA and $p75^{NTR}$, proNGF is neurotrophic, but if TrkA levels are reduced or absent, proNGF exhibits apoptotic activity via $p75^{NTR}$ and sortilin [37]. Therefore, the balance between apoptotic and neurotrophic signaling is achieved by receptor expression.

Pro-Neurotrophin Receptors and Downstream Signaling

While BDNF binds preferentially to TrkB and activates neurotrophic pathways, proBDNF binds preferentially to $p75^{NTR}$ and activates intracellular signaling pathways that mediate synaptic weakening and apoptosis. $p75^{NTR}$ activation activates the RhoA- Rho-associated protein kinase (ROCK) pathway which reduces dendritic spine stability and mobility [33]. $p75^{NTR}$ also activates JNK, leading to the phosphorylation of the c-Jun transcription factor and subsequent transcription of proapoptotic genes [33].

The functional outcome of proNGF binding, on the other hand, is contingent on the receptor complement of the target cell. In the absence of TrkA and in the presence of sortilin and $p75^{NTR}$, proNGF is apoptotic. In the presence of TrkA, proNGF signaling results in the activation of Ras-MAP kinase, PLCγ, and PI3 kinase/Akt pathways, leading to apoptotic suppression, neurite outgrowth, and cell survival [33]. The ability of proNGF to bind to both TrkA and $p75^{NTR}$ accounts for its flexibility in signaling and undoubtedly contributed to the initial confusion surrounding its true function.

NGF, proNGF, and Neuronal Refinement

The role of NGF and proNGF in development has largely been limited to studies of the peripheral nervous system where the trophic hypothesis of development was initially generated. It is widely accepted that sympathetic neurons in the periphery that are responsible for nociception and dorsal root ganglion (DRG) neurons in the spinal cord rely on NGF during development for proper target innervation. Withdrawal of NGF in either cell culture models or *in vivo* through the removal of synaptic targets results in the release of cytochrome c, a proapoptotic regulator, from mitochondria, leading to cell death via activation of caspases [38]. While the role of mature NGF in the peripheral nervous system has been studied for decades, recent literature suggests that, as it is in the CNS, proNGF is the critical isoform in the periphery. Western blots of NGF-sensitive tissues in the periphery including the trigeminal ganglion and the superior cervical ganglion demonstrate robust proNGF expression in the absence of mature NGF expression. While these peripheral tissues may be responsive to mature NGF, the ligand responsible for endogenous neurotrophic activity during development appears to be proNGF.

An important factor to appreciate in the periphery is the long distance between sympathetic cell bodies and their tar-

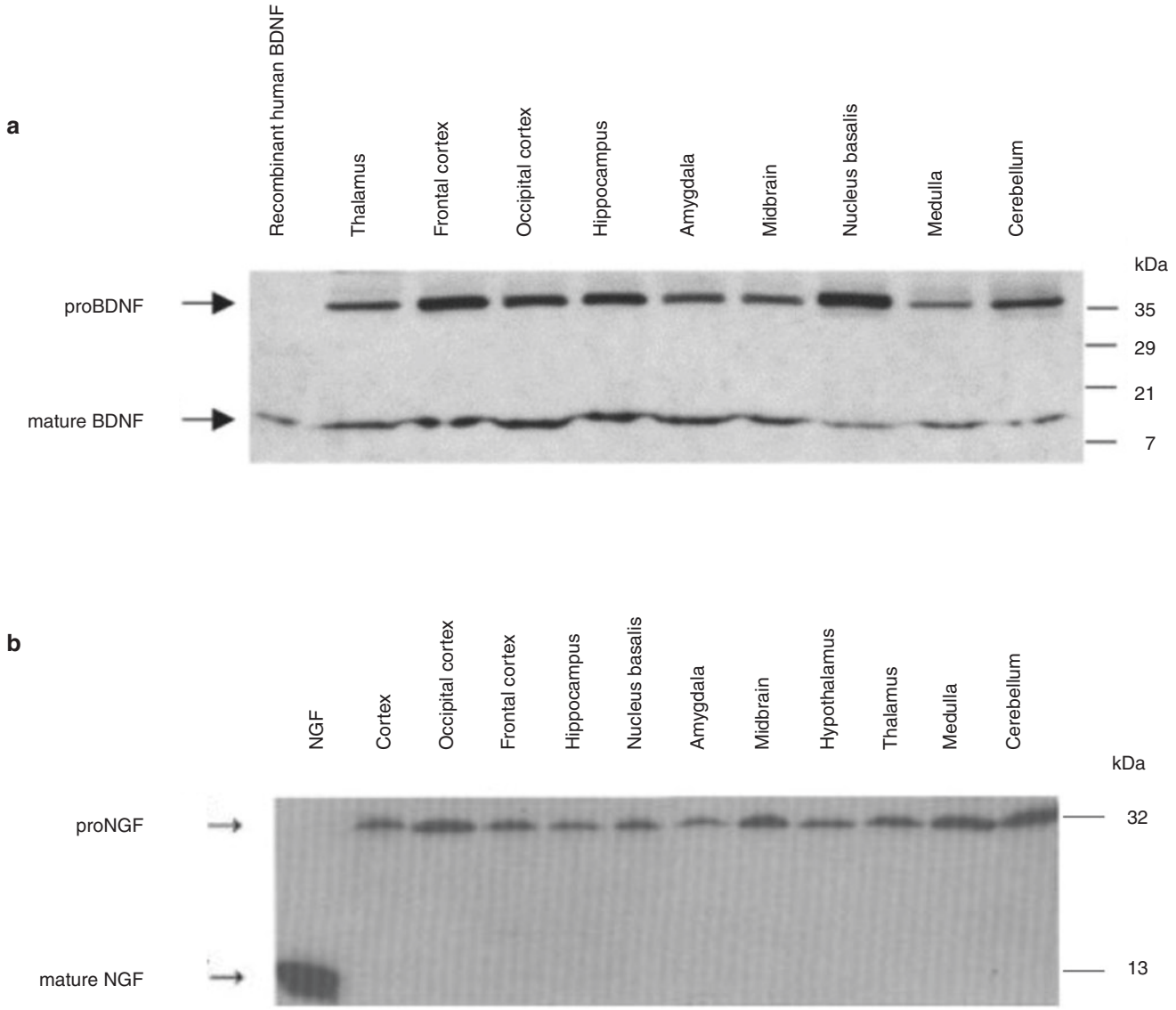

Fig. 4.6 Western blots probing for BDNF (**a**), NGF (**b**) and their respective precursor isoforms in different areas of the brain. BDNF and proBDNF are both found throughout the brain, while NGF is found only as proNGF. Michalski & Fahnestock 2003 for Fig. 4a. Fahnestock et al. 2001 for Fig. 4b

gets. Target-derived trophic factors can signal over these long distances via retrograde axonal transport of TrkA-containing signaling endosomes to the cell body [39]. Neurotrophins binding to Trk receptors trigger internalization of the ligand–receptor complex into signaling endosomes that are transported along microtubule networks in the axons via energy-dependent dynein motor proteins. Although still controversial, evidence supports the model that proNGF is the NGF isoform that binds, is internalized, signals and is transported intact to the cell body [36, 40–42]. While the delivery of the TrkA-proNGF signaling endosome to the cell body is important, the complex continually signals as it travels along the axon, providing local survival signaling to maintain axon integrity within the long projections themselves [39].

While most of the focus on the role of NGF/proNGF during development has been in the periphery, it does play a limited role in the brain. Both striatal interneurons and basal forebrain cholinergic neurons express TrkA [43]. Cultured basal forebrain neurons from developing murine and avian embryos are heavily reliant on BDNF and NGF isoforms for their proper function [44]. Specifically, trophic support is important for the development of the exceptionally long and diffuse neuronal processes that are characteristic of cholinergic neurons in the basal forebrain.

Neurotrophins and Development

Neurotrophins and their respective Trk receptors are necessary for normal development in both the central and peripheral nervous systems. The trophic hypothesis attempts to

explain why so many neurons die after their axons reach their targets. As previously mentioned, survival of developing neurons is dependent upon neurotrophins secreted by their targets. Targets secrete limited amounts of neurotrophins, resulting in competition between innervating neurons and survival of only those neurons sufficient to satisfy the needs of their respective target fields [13] (Fig. 4.2).

NGF

While we know that endogenous NGF exists primarily as the precursor proNGF, previous experiments have been done using mature NGF, and so we will use the term NGF in this section to refer to either mature NGF or unprocessed proNGF [36, 45]. In rodent studies, TrkA and NGF first appear around embryonic day (E)10.5–11 in concert with an increase in NT-3 and BDNF levels that correlate with mass neurogenesis [11]. TrkA expression levels peak slightly later, with 80% of neurons expressing TrkA by E15. The peak in TrkA at E15 coincides with increases in NGF levels when neurons finally reach and interact with their innervating targets [46]. Many PNS neurons are dependent on NGF for survival during development and afterwards. NGF is necessary for the survival and differentiation of the dorsal root and trigeminal ganglia sensory neurons as well as sympathetic neurons of the PNS such as the superior cervical ganglion [47]. For example, nociceptive neurons do not fare well when deprived of NGF. In the DRG and trigeminal ganglia, as many as 80% of sensory neurons die when faced with NGF deprivation. NGF is synthesized by many target tissues such as the heart, skin, submandibular gland, pineal gland, and extracerebral blood vessels [45]. Retinal ganglion cells, bipolar cells, cells of the endothelium, and smooth muscle as well as fibroblasts and myofibroblasts are reported to be both receptive to NGF (contain NGF receptors) and produce NGF. NGF also plays a role in the immune system. Lymphocytes, mast cells, and eosinophils all secrete NGF [48].

While most of the focus on the role of NGF during development has been in the periphery, it does play a limited role in the brain due to the expression of TrkA on certain CNS neurons such as basal forebrain cholinergic neurons and striatal cholinergic interneurons. Basal forebrain cholinergic neurons have long, extremely diffuse projections, forming synaptic connections with almost all areas of the cortex and the hippocampus. NGF is synthesized mainly in pyramidal neurons of the cortex and hippocampus. NGF that is released at the synapse binds TrkA on the postsynaptic basal forebrain neuron. The NGF-TrkA complex is then internalized and the now-activated TrkA can signal locally to support axonal survival and distally to the cell soma while being retrogradely transported in signaling endosomes [49]. Basal forebrain cholinergic neurons are dependent on NGF signaling to promote cellular differentiation (regulation of cholinergic enzymes) and survival. Differentiation, regulation, and survival of many peripheral and some central neuronal populations are dependent on NGF, while populations which are not NGF-dependent require the presence of other neurotrophins [33].

BDNF

During development, the earliest reported evidence of BDNF expression is in the neural tube, which contains migrating BDNF-responsive neural crest cells [50]. BDNF follows NT-3 as the second neurotrophin to appear in early development. *In vitro* studies of neural progenitor cells in culture found that BDNF is necessary for proliferation and differentiation of these cells. BDNF levels increase coincident with mass embryonic neurogenesis in both the PNS and CNS [50].

BDNF drives differentiation of neural crest-derived cells to a sensory neuron lineage during early embryonic development and is critical for the survival of many sensory neuron populations [51]. Many studies have created knockout mouse models to examine the role of neurotrophins in development. Heterozygous BDNF knockout mice present with no severe deficits and can live over a year. The homozygous BDNF knockout, however, is lethal with most mice dying soon after birth due to malformations of the heart, although a small number survive for several weeks [52].

In the PNS, sensory neuron support is shared among neurotrophins. BDNF and NT-3 support the survival of vestibular and cochlear ganglia responsible for balance, locomotion, and hearing. Knocking out BDNF alone causes severe deficits in movement and coordination but not a complete loss of neurons [53]. NT-3/BDNF knockout mutant mice, however, show loss of the entire ganglia and die quickly after birth [54]. These experiments demonstrate that NT-3 and BDNF together support these ganglia. Nodose-petrosal ganglia, however, which are responsible for regulating heart rate, breathing rate, and gut motility, depend on BDNF and NT-4 [11, 55]. Despite this fact, BDNF knockout mice that survive past the first 48 hours after birth develop irregular breathing, while NT-4 knockout mice do not, suggesting that only BDNF, and not NT-4, is essential for supporting a subset of afferents involved in breathing regulation in newborn mice [52]. Another example of shared target-derived trophic support is the DRG. Subpopulations of DRGs are not only supported by NGF, but also by BDNF, NT-3, and NT-4 [56]. Loss of support from one neurotrophin results in the loss of a subpopulation of DRG neurons, but not all. Taken together, neurotrophin support in the periphery during development is shared between BDNF, NGF, NT-3, and NT-4, with most sensory ganglia showing a selective loss of specific neuronal subpopulations when individual neurotrophins are knocked out.

In the CNS, BDNF is involved in cell proliferation, migration, and differentiation of certain neurons during early embryonic development. BDNF knockout mice exhibit minimal neuronal loss in the CNS, with a few exceptions [57]. Interestingly, BDNF, NT-3, and NT-4 are all able to individually support survival of subsets of motor neurons in culture; however, no one neurotrophin is able to support survival of all motor neurons *in vivo*. BDNF, NT-3, and NT-4 triple-knockout mice exhibit only a 20% loss of facial and spinal motor neurons, leaving the majority unaffected [11, 56]. This suggests that there are subpopulations of motor neurons which rely on different trophic factors for support. In addition, the proliferation of basal forebrain ɣ-aminobutyric acid (GABA)-ergic neuron precursor cells depends on BDNF support. Mesencephalic dopaminergic neurons in the substantia nigra and magnocellular cholinergic neurons in the basal forebrain are dependent on BDNF for differentiation during development [57]. BDNF is important for the survival of cerebellar granule cells and their migration from the proliferative zone (the external granule cell layer) to their target destination in the internal granule cell layer [58]. Hippocampal and cortical neurons require BDNF support for differentiation, although the lack of hippocampal and cortical loss in BDNF knockout animals suggests that these neurons do not depend on BDNF for survival [57].

BDNF is the most highly expressed neurotrophin in the postnatal brain, and postnatal BDNF expression is essential for the continued development and refinement of specific neuronal connections, adult neurogenesis, and synaptic plasticity. BDNF levels are the highest in the cortex and hippocampus. During postnatal development, changes in BDNF levels are temporally dependent and species and region specific. In the rat hippocampus, BDNF levels steadily increase and then plateau at 4 months with levels 20 times higher than on postnatal day (P) 0. In the macaque monkey, hippocampal BDNF levels peak after the first postnatal year and then slowly decline with age. Similarly, in humans, hippocampal BDNF levels continue to rise postnatally, plateau a few years after birth, remain elevated, and then slowly begin to decline in old age [59]. This rise after birth is consistent with the continued postnatal development of hippocampal neurons. Human BDNF expression is the highest in the hippocampus and cortex postnatally most likely because of BDNF's essential role in adult neurogenesis, synaptic plasticity, and learning and memory formation [59].

In other parts of the brain, BDNF levels show similar trends. For example, in human tissue studies, BDNF mRNA levels increase in the dorsolateral prefrontal cortex (DLPFC) in infants, reaching the highest levels in young adults and remaining elevated throughout adulthood [60]. This is in line with the postnatal development of the cortex which does not reach its functional maturity until adulthood. This postnatal increase in BDNF is believed to correlate with synaptic plasticity and neuronal maturation that takes place during cortical development [60]. While many neurons in the brain depend on BDNF for differentiation, maturation, and synaptic connectivity, they do not depend on BDNF for survival [57]. Taken together, BDNF is a pleiotropic neurotrophin that is an essential mediator of development necessary for embryonic and postnatal neurogenesis, the survival of distinct sensory neuron populations, cell proliferation, differentiation, migration, arborization, and formation of appropriate synaptic connections.

BDNF, Activity-Dependent Expression, and Dendritic and Axonal Arborization

While BDNF is involved in neurogenesis, differentiation, and migration, it is also required for different aspects of neuronal development such as dendritogenesis, spinogenesis, and axonal arborization. Deletion of BDNF's receptor, TrkB, in new neurons (neuroblasts) in the hippocampus and the olfactory bulb stunts dendritogenesis and impairs spine formation [61]. Conversely, *in vivo* treatment with BDNF induces growth and increases complexity of axonal and dendritic arbors in retinal ganglion cell axons and optic tectum of *Xenopus* tadpoles [62].

Classic studies performed in the monkey and cat visual cortex led to the discovery that activity-dependent competition for trophic support is essential for the formation of ocular dominance columns in the visual system [63, 64]. In the visual cortex, ocular dominance columns are stripes, or columns, of segregated lateral geniculate nucleus (LGN) inputs from the thalamus that project into layer IV of the visual cortex. They are important for binocular vision and depth perception in many animals such as cats, primates, and humans [63]. The columns represent distinct inputs from each eye that have not yet been integrated. In these initial studies, monocular deprivation of light impeded segregation of lateral geniculate nucleus axons into columns in the primary visual cortex (layer 4) of the deprived eye. It was later determined that activity-dependent competition for trophic support is essential for the formation of ocular dominance columns in the visual system [65]. Layer-specific, dynamic expression of BDNF and NT-3 is required for regulation of dendritic arborization and axonal branching and subsequent segregation of ocular dominance columns [66]. BDNF and NT-3 expression levels change throughout different stages of development and during the critical period in the visual system in response to activity. The critical period is a postnatal time period when visual input modifies synaptic development, giving rise to ocular dominance columns. For example, BDNF is found initially, before eye opening, at very low levels and only in layers V and VI of the visual cortex. After eye opening, these levels rise and then are found in layers II and III as well. Thereafter, BDNF is maintained at higher levels throughout adulthood. Conversely, NT-3 levels are only detectable in the visual cortex after eye opening and

slowly decline by the end of the critical period. BDNF's regulation of dendritic and axonal branching is region and layer specific, however, with BDNF driving dendritic growth and arborization in some layers but inhibiting it in others. More specifically, BDNF increases dendritic growth in layer IV pyramidal neurons of the cortex and inhibits it in layer VI. Conversely, NT-3 inhibits growth and dendritic arborization in layer IV and stimulates it in layer VI [66]. This antagonism in the regulation of dendritic growth of pyramidal neurons in the visual system during development suggests that BDNF and NT-3 work together in a reciprocal signaling system to regulate the growth and branching of dendrites needed for synapse formation. This mechanism, sometimes referred to as a "push and pull" mechanism, demonstrates that the function of neurotrophins can be layer- and cell-specific [66]. Interestingly, application of exogenous BDNF or NT-4 or TrkB-IgG, which binds BDNF and NT-4, prevented this effect, suggesting that competition of endogenous ligands specific for TrkB is required during the critical period of development for the ocular dominance columns [67]. Sensory stimulation of the visual system increases activity-dependent release of BDNF and enhances TrkB activity, which increases the maturation of inhibitory interneurons which are required for ocular dominance column formation [68]. Interestingly, in addition to this, BDNF also stimulates the maturation of excitatory neurons.

BDNF and Synapse Development

BDNF is required for the formation and maturation of both glutamatergic (excitatory) and GABA-ergic (inhibitory) synapses throughout development [69]. BDNF and glutamate act in concert to regulate excitatory synaptic transmission. In the hippocampus and cerebral cortex, BDNF acutely increases excitatory synaptic transmission. Specifically, BDNF induces glutamate release presynaptically. BDNF also enhances mini excitatory postsynaptic currents (mEPSCs) in cultured hippocampal neurons by increasing the probability of N-methyl-D-aspartate receptor (NMDA) receptor opening frequency. BDNF can achieve this by phosphorylating tyrosine residues on NR1 and NR2B NMDA receptor subunits on the postsynaptic cell. While there is some controversy as to whether BDNF-led synaptic potentiation is mediated primarily by presynaptic or postsynaptic mechanisms, research has found evidence for both [70].

In addition to its effects on excitatory synapses, BDNF also regulates GABAergic synapse formation and maturation during development (Fig. 4.7). While in adulthood GABAergic signaling is inhibitory, in the developing

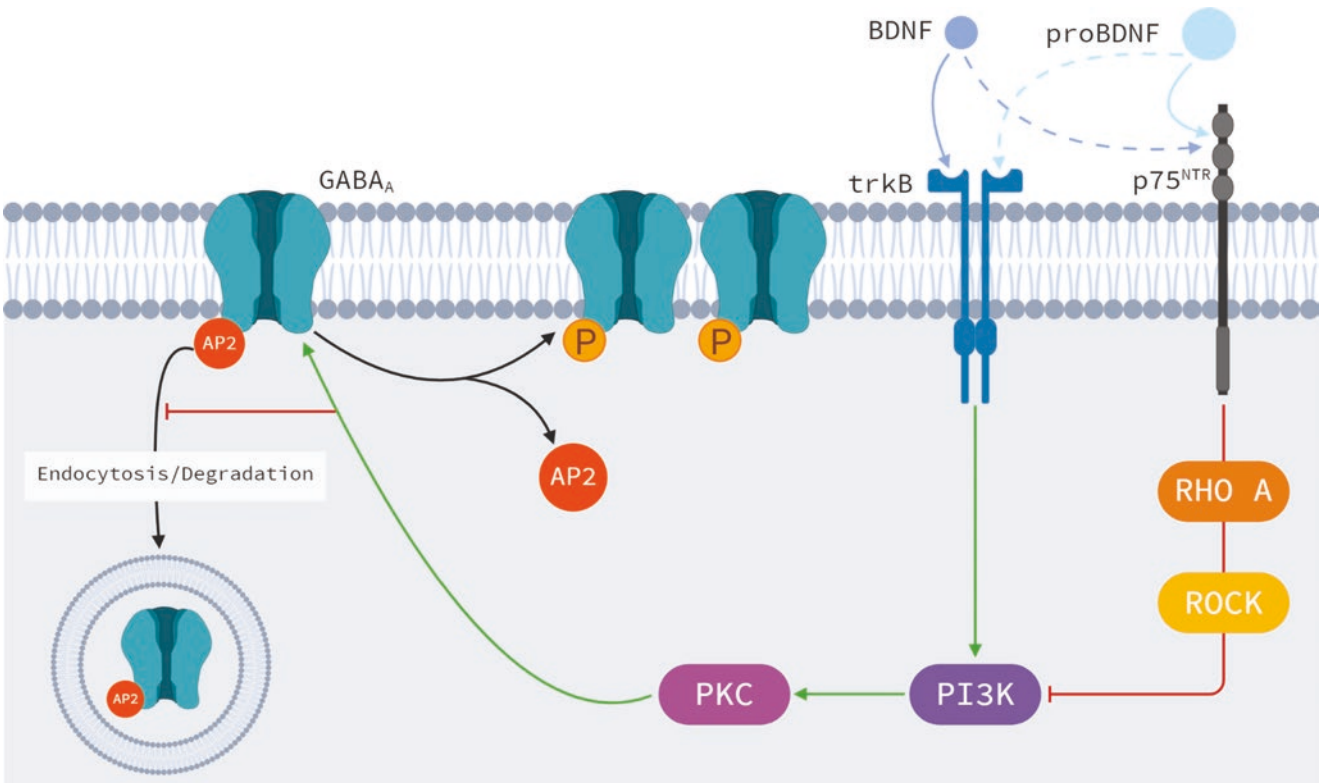

Fig. 4.7 Regulation of ionotropic GABA$_A$ receptors by BDNF and proBDNF during development. BDNF-TrkB binding inhibits GABA$_A$ R endocytosis through the PI3K and PKC signaling pathways, increasing the expression of GABA$_A$ receptors on the postsynaptic cell, strengthening GABAergic signaling. Conversely, proBDNF-p75^{NTR} binding can dephosphorylate GABA$_A$ R through the RhoA/Rock pathway, leading to internalization and degradation of GABA receptors. The balance between pro and mature BDNF signaling is critical for the regulation of GABAergic synapses during development

CNS, GABAergic signaling results in excitatory activity [70, 71]. This can be attributed to a higher intracellular Cl^- concentration (high $[Cl^-]_i$) in immature neurons. The neurotransmitter GABA binds to two receptor types, $GABA_A$ and $GABA_B$ receptors ($GABA_AR$ and $GABA_BR$). $GABA_AR$ signaling is ionotropic, while $GABA_BR$ signaling is metabotropic. Ionotropic $GABA_A$ receptors allow any anions (but particularly chloride ions [Cl^-]) to pass bidirectionally in and out of the cell. The direction of ion flow (influx or efflux) depends on the intracellular Cl^- ion concentration. During adulthood, $GABA_A$ receptor activation during depolarization of a neuron results in an influx of Cl^- ions. This influx of negatively charged ions weakens depolarization (hyperpolarizes) of the cells [69]. As a result, the inhibitory postsynaptic potentials (IPSCs) prevent firing of action potentials. In the developing brain, due to a higher intracellular Cl^- resting level, this effect is reversed, with $GABA_A$ R activation producing Cl- efflux and thus, depolarization. Both mature BDNF (mBDNF) and proBDNF regulate this process by regulating GABA receptor density on the postsynaptic cell. mBDNF-TrkB binding inhibits $GABA_A$ R endocytosis through the PI3K and PKC signaling pathways and as a result increases the expression of $GABA_A$ receptors on the postsynaptic cell, strengthening GABAergic signaling [69]. mBDNF-TrkB also increases GABA receptor expression [69]. Conversely, proBDNF-$p75^{NTR}$ binding can dephosphorylate $GABA_A$ R through the RhoA/Rock pathway, leading to internalization and degradation of GABA receptors, providing further evidence that together, mature and pro BDNF regulate the maturation and efficacy of GABAergic synapses [69].

Cl^- intracellular levels are regulated by two chloride cotransporters, the potassium chloride cotransporter 2 (KCC2) and the Na-K-Cl cotransporter (NKCC1). NKCC1 leads to an accumulation of Cl^- inside the cell, whereas KCC2 is responsible for Cl^- extrusion. KCC2 is expressed later in development, while NKCC1 is more abundantly expressed in early development. The expression pattern of these chloride cotransporters explains why immature neurons have higher $[Cl^-]_i$ than mature neurons. BDNF also regulates the expression of KCC2 throughout development, suggesting that BDNF is involved in the establishment of these GABAergic synapses and the shift to their canonical inhibitory role [71]. These findings demonstrate that BDNF is essential during development for the establishment and regulation of excitatory synapses, inhibitory synapses, and the balance between them.

BDNF and Synaptic Plasticity

Synaptic plasticity is a dynamic process that balances axonal and dendritic growth and retraction. Refinement of neural circuits is the result of neural activity caused by sensory or motor stimulation or spontaneous electrical activity. This results in the strengthening of some circuits and the weakening of others. Activity-dependent synaptic plasticity is regulated by neurotrophic factors. BDNF is necessary for the regulation of processes such as synapse formation, dendritic growth, and spine maturation, all of which are important for synaptic plasticity, which is the basis for learning and memory formation in the brain.

The most studied form of synaptic plasticity is hippocampal long-term potentiation (LTP) which depends upon BDNF. LTP is produced when repeated stimulation (high frequency stimulation) is applied to a neuron resulting in either short or prolonged increases in synaptic efficacy and is believed to be the underlying mechanism of learning and memory formation. LTP can be separated into two temporally distinct phases, early phase (E-LTP) and late phase (L-LTP). L-LTP is initiated via strong, high-frequency stimulation, and the resulting increases in synaptic efficacy can last several hours or even days. L-LTP induces an influx of calcium into the cell via voltage-gated calcium channels or NMDA receptors. L-LTP results in the production of new mRNA and synaptic structural changes, both of which are believed to be required for long-term memory formation [72, 73]. E-LTP, on the other hand, is initiated by weak, high-frequency stimulation and is considered a short-lasting form of LTP. E-LTP results in increases in synaptic efficacy for 1–2 hours. E-LTP does not require new protein production, but instead involves the trafficking of existing protein to activated synapses.

It is well established that BDNF is required for LTP and that, in turn, LTP can induce BDNF expression. Secretion of pre-existing BDNF protein is essential for E-LTP. A single nucleotide polymorphism in the BDNF gene that produces BDNF protein containing a valine to methionine substitution at codon 66 (known as val66met) reduces BDNF secretion. This polymorphism impairs short-term episodic memory, which depends upon E-LTP.

BDNF is also an important component of L-LTP. L-LTP increases the expression of kinases that phosphorylate transcription factors such as CREB and E26 transformation specific-like protein 1 (Elk-1). The activation of these transcription factors induces expression of new mRNAs which leads to changes at the synapse. In particular, L-LTP results in the transcription of the BDNF gene and secretion of BDNF, which in turn binds TrkB on either the pre- or postsynaptic cell. BDNF protein activates TrkB and its signaling pathways PI3K, Ras-MAPK, and Rac, resulting in actin cytoskeletal remodeling, protein synthesis, and postsynaptic density protein 95 (PSD-95) trafficking, which are all involved in synapse formation and maturation and synaptic plasticity [72].

These changes at the synapse as a result of E-LTP and L-LTP correlate with short-term and long-term memory formation, respectively. In both BDNF heterozygous and homozygous knockout mice, LTP is reduced and animals show

impairments in memory consolidation and acquisition [74]. Similarly, TrkB knockout mice also exhibit impairments in LTP [75]. These studies demonstrate that BDNF and TrkB are essential for learning and memory formation, LTP, and synaptic plasticity.

Synaptic plasticity involves both the strengthening of synapses through LTP and the weakening of synapses via long-term depression (LTD). Just as high-frequency stimulation increases synaptic efficacy through LTP, prolonged low-frequency stimulation induces synaptic weakening through LTD [76]. LTD weakens synaptic connections in order to make way for new memories to be stored through LTP. Without this process, LTP would reach a plateau, thereby limiting the amount of new information that could be processed and stored. The balance between synaptic strengthening via LTP and synaptic weakening via LTD is critical for learning and memory formation [30, 72].

While hippocampal LTP is heavily regulated by BDNF/TrkB binding, its counterpart, LTD, is regulated by proBDNF binding to $p75^{NTR}$. Mature BDNF binding to TrkB strengthens synapses and increases axonal and dendritic arborization associated with LTP, while proBDNF binding to $p75^{NTR}$ weakens synapses, lowers spine density, and decreases dendritic complexity implicated in LTD. Similar to LTP, LTD can also involve NMDA receptors. LTD can be both NMDAR-dependent and independent. Specifically, proBDNF/$p75^{NTR}$ signaling governs NMDA-dependent forms of LTD; proBDNF binding to $p75^{NTR}$ increases the expression of the NMDA receptor subtype 2B (NR2B), facilitating NMDAR-specific LTD [30]. In support of this, low-frequency stimulation applied to hippocampal slices of $p75^{NTR}$ knockout mice fails to elicit NMDA-dependent LTD [30]. Treatment with recombinant cleavage-resistant proBDNF increases LTD in wild-type mouse hippocampal slices, and cleavage-resistant proBDNF knock-in mice have increased LTD, reduced dendritic complexity and decreased LTP in the hippocampus [76]. The refinement of neural circuits during development depends upon activity-dependent modulation of BDNF and proBDNF, which invokes both LTP and LTD.

NT-4

While NT-4 also binds both TrkB and $p75^{NTR}$, its role in development is distinct from that of BDNF. Interestingly, NT-4 and BDNF are secreted from different target subpopulations in the periphery. Their non-overlapping pattern of secretion may be required for the recognition of correct targets. For example, BDNF is responsible for one subpopulation of nodose-petrosal ganglion neurons and NT-4 another [11]. This is also true in the trigeminal mesencephalic nucleus and the DRGs where NT-4, BDNF, and NT-3 are essential for the survival of different subpopulations of neurons. Interestingly, while they are similar in structure and bind to TrkB with similar affinities, BDNF and NT-4 appear to have functionally distinct physiological roles. For example, intracortical infusion of BDNF changes electrophysiological activity, while NT-4 infusion does not. This may be due to different activation or endocytic sorting of TrkB by BDNF versus NT-4, leading to activation of different pathways of signal transduction or different time courses of downstream signaling [77, 78]. For example, TrkB point mutations that impair SHC adaptor protein binding exhibit reduced response to NT-4 but not to BDNF. NT-4 induces less rapid degradation of TrkB than BDNF, leading to more sustained downstream signaling [79]. Thus, NT-4 and BDNF share many roles yet are functionally, spatially, and temporally distinct.

NT-3

NT-3 mRNA can be detected in avian and mammalian embryos as early as the first embryonic day (E1) [38]. NT-3 is the most highly expressed neurotrophin by E12, suggesting that it plays a critical role during development. A subset of TrkC-expressing progenitor cells from the neural crest is responsive to NT-3 during development [80]. Many of the progenitor cells from the neural crest that are responsive to NT-3 develop into neurons that populate the enteric nervous system (ENS). The ENS is a division of the autonomic nervous system and is responsible for the function of the gastrointestinal tract. While NT-3 is critical during development and does play a role in some postnatal functions, its expression in the ENS is reduced postnatally.

ENS neurons are uniquely responsive to NT-3 during development; this is corroborated by the presence of TrkC but the absence of both TrkA and TrkB in the developing ENS [81]. Addition of NT-3 to E14 neurons isolated from fetal rat gut results in the extension and arborization of their neurites [82]. ENS neurons grown in the absence of NT-3 or in the presence of either an NT-3 antibody or TrkC antagonist do not develop proper synaptic connections, suggesting that NT-3 is critical for the development of proper connectivity in the ENS [80]. Both embryonic and postnatal ENS neurons undergo apoptosis upon withdrawal of NT-3 [82]. Mice that are genetically engineered to overexpress NT-3 display hyperplasia of enteric ganglia due to the presence of larger neurons and increased dendritic branching [80]. Furthermore, transgenic mice that do not express NT-3 or TrkC display severe deficits in neuronal density of many enteric ganglia, suggesting that NT-3 regulates the survival of ENS neurons during development [80].

It is important to note that the development of the ENS is also contingent on other neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF), and that the dependence of ENS neurons on specific neurotrophins

changes as development progresses [83]. Pluripotent cells destined to become ENS neurons are initially responsive to GDNF, which triggers a massive wave of proliferation, allowing eventual ENS neurons to colonize the entirety of the gut. After E12, GDNF-mediated proliferation ceases and ENS precursor cells begin to express bone morphogenic protein (BMP) 2 and 4, causing the arrest of the cell cycle and greatly increased expression of TrkC. After E14, ENS neurons no longer depend on GDNF and require NT-3 to promote their survival and neurite outgrowth. While NT-3 expression does significantly decrease postnatally, it still plays a role in the maintenance of the adult ENS. NT-3 is responsible for the continued survival of myenteric and submucosal neurons in the adult ENS.

While NT-3 has been most extensively studied in the context of ENS development, it also plays a role in the development of other cell types, including oligodendrocytes, and mediates the vascular coupling of peripheral nerves during development [84]. Oligodendrocytes are glial cells that produce myelin and myelin basic protein (MBP), the principal components of the myelin sheath that envelops certain axons in order to facilitate efficient signal conduction. NT-3 is critical for the differentiation of pluripotent stem cells into oligodendrocytes. Exposure to NT-3 triggers early oligodendrocytes to produce significant amounts of MBP [85]. Defective neuronal myelination is the hallmark symptom of multiple sclerosis (MS), making NT-3 a potential therapeutic target for the disorder.

Finally, the prenatal expression of NT-3 by blood vessels is important for sympathetic nerve pathfinding in the periphery [86, 87]. Developing axons grow along NT-3-producing blood vessels en route to their targets. While NT-3 is by no means the sole guiding factor that promotes this interaction in the periphery, NT-3 knockout mice display clear deficits in sympathetic innervation during development. This is most evident in cardiac-projecting axons that originate within the stellate ganglion in the cervical vertebrae [84]. Sympathetic cardiac innervation from this ganglion is established by E16.5 in wild-type mice but is completely absent in NT-3 knockout mice. Similar NT-3-mediated innervation deficits can be observed in other peripheral targets including Merkel cells in the skin that are responsible for mechanoreception [88]. The ability of blood vessels to act as guiding intermediate targets for pathfinding axons via NT-3 expression plays a key role in PNS development.

Parallels in the Adult Brain

Neurogenesis

At the onset of postnatal life, most of the nervous system permanently arrests proliferation and remains post-mitotic for the duration of the lifespan. However, two areas in the CNS, the subventricular and subgranular zones, continue to generate new neurons through a process termed adult neurogenesis [89]. Proliferation in these regions mirrors development, as newly generated neurons are produced in excess and many undergo apoptosis. New neurons produced in this area are integrated into the hippocampus. Inhibiting either postnatal neurogenesis or neuronal apoptosis in the subventricular zone impairs learning and memory, suggesting that postnatal neurogenesis and apoptosis work in tandem to refine the connectivity of the adult brain to facilitate learning and memory [90]. The fate of new neurons in the postnatal nervous system is regulated largely by neuronal activity, making activity-dependent BDNF a key regulatory molecule in the survival and integration of newly generated neurons in the brain [91]. NT-3 also plays a role in postnatal nervous system development due to the existence of NT-3 responsive oligodendrocyte progenitors within the adult CNS parenchyma [85]. While NT-3 can be used to trigger MBP production in postnatal animals, the functional consequences of the remyelination that occurs via this mechanism are currently unknown.

BDNF and Neurodevelopmental Disorders

The nervous system requires a complex series of sequences for proper development. Even slight alterations in this delicate balance of synaptic growth and refinement leave the nervous system vulnerable to perturbations that could lead to permanent abnormalities. It has been well established that neurotrophin expression during development is region, tissue, and stage specific. Disruptions in the intricately timed expression patterns of neurotrophins during development underlie many neurodevelopmental conditions, some of which will be explored in this section.

Autism Spectrum Disorder

The mammalian target of rapamycin (mTOR) pathway is a downstream effector of BDNF-TrkB signaling whose disruption underlies the symptoms of Autism Spectrum Disorder (ASD) [92, 93]. ASD is a lifelong developmental disorder that is characterized by abnormal social behavior, repetitive or restricted behavior, and impaired communication [94]. ASD may arise from defective synaptic development and plasticity. It is believed that abnormal synaptic function adversely affects the development and maintenance of neuronal connections in the ASD brain, thus contributing to autism-related behavioral deficits. While there is a strong link between ASD and genetics, there are also cases of "idiopathic" autism that have no known genetic cause and may be due to epigenetic mechanisms or environmental factors such

as infection, pesticide, or drug exposure *in utero* [95]. Genetic and epigenetic studies have implicated molecules involved in synaptic development and plasticity in ASD such as those in the mTOR pathway [96]. These molecules are important for synaptic development and plasticity due to the downstream signaling pathways they activate which regulate protein synthesis and motility at dendritic spines. Studies have found higher levels of proBDNF protein in subjects with autism and lower levels of full-length TrkB, resulting in decreased signaling through the Akt-mTOR pathway in idiopathic autism [93]. Additionally, genetic studies have found that single nucleotide polymorphisms (SNPs) of the BDNF and TrkB genes are linked to ASD.

Genetic evidence also supports mTOR pathway disruption as a key underlying cause of autistic symptoms. Single-gene disorders with high rates of autism, such as Fragile X, tuberous sclerosis, and macrocephaly involve mutations in proteins of the Akt-mTOR pathway resulting in overactivation of downstream signaling. Similarly, underactivation through this pathway such as in Rett syndrome reduces spine protein synthesis and could therefore affect synaptic development and maintenance of neuronal connections that are important for normal development [96]. It is clear that any disruption (under- or over-activation) in the mTOR pathway causes synaptic dysfunction underlying ASD, and that imbalances in BDNF and TrkB isoforms may alter signaling through this pathway.

Epilepsy

Temporal lobe epilepsy (TLE) is a progressive form of seizures characterized by increasing seizure severity and frequency, medical intractability, degeneration of cortical tissue, and cognitive impairment [97]. Both NGF and BDNF mRNA and protein expression are increased in the hippocampus following seizure and contribute to seizure progression. *In vitro* studies report hyperexcitability after direct administration of BDNF to hippocampal neurons in culture, and in mice, intraventricular or intrahippocampal infusion of BDNF or overexpression of BDNF or TrkB increases seizure susceptibility or severity. Similarly, intraventricular administration of NGF increases seizure progression in kindling models (a model of epilepsy in which repetitive electrical stimulation of forebrain structures can induce permanent seizure susceptibility). In contrast, blocking NGF or TrkA action by administering blocking antibodies or peptides, or conditional knockout of TrkA, eliminates epileptogenesis [97, 98]. Taken together, this evidence suggests that increased expression of NGF and BDNF following seizure enhances the development of epilepsy.

Similar to what is found during early development, GABA receptor activation in epilepsy is excitatory [99]. As in development, this is due to an increased accumulation of intracellular Cl^- ions. Recurrent seizures lead to an increase in intracellular Cl^- ions. As a result of this intracellular Cl^- buildup, Cl^- ion efflux renders the cell less negative and brings the graded potential of the cell closer to the threshold (~-55 mV), making it more likely to fire an action potential. This explains why increases in BDNF and NGF contribute to epileptogenesis, as both are known to increase excitatory synaptic transmission. Seizures also cause BDNF-induced activation of TrkB in the mossy fiber pathway of the hippocampus, which may increase GABA release. The immature brain is more vulnerable to seizures than the adult brain for many reasons, one being its higher intracellular Cl^- levels, with GABAergic signaling producing Cl^- efflux and depolarization. In adulthood, where intracellular Cl^- levels are lower and GABA is inhibitory, GABA antagonists induce seizures and GABA agonists prevent seizures [99]. Overall, a fine balance between excitatory and inhibitory signaling must be maintained to prevent hyperactivation and the development of epilepsy.

Fetal Alcohol Spectrum Disorder

BDNF and NGF are the two neurotrophins most heavily impacted by alcohol exposure during development. In rodents, prenatal exposure to alcohol results in decreased BDNF levels in the adult medial prefrontal cortex, cortex, and hippocampus. Additionally, in some studies, prenatal alcohol exposure decreases TrkB expression in the newborn male rat hippocampus. Decreased BDNF and TrkB can negatively impact dendritic morphology and complexity, neuronal integration, and signal transduction. Other studies found that prenatal alcohol exposure in rats did not alter the amount of TrkB but decreased TrkB phosphorylation in the hippocampus, suggesting that prenatal alcohol exposure affects BDNF-TrkB signaling. And while many studies report that TrkB levels eventually return to baseline following prenatal alcohol exposure, alterations in the tightly controlled expression patterns of BDNF and TrkB during critical stages of development can lead to developmental issues such as impairments in neuroplasticity and learning and memory formation [100]. In adult rodent studies, NGF levels increase shortly after alcohol exposure. During development however, fetal alcohol exposure decreases NGF and TrkA expression in the hippocampus and cerebellum of male rat pups and increases NGF expression in the cortex. These changes to NGF expression can be detrimental to neurons that require NGF for survival throughout development [100].

Alcohol exposure also causes alterations in signaling proteins downstream of NGF and BDNF. For example, ERK and PIK3 protein expression is inhibited after prenatal alcohol exposure in cerebellar granule neurons during develop-

ment. Similarly, alcohol exposure decreases MAP and Akt signaling pathway proteins in the cortex [100].

Glutamatergic activity increases BDNF and proNGF levels in the hippocampus. Alcohol acts as an NMDA glutamate receptor antagonist, and therefore reduced BDNF and proNGF expression following alcohol exposure is likely mediated indirectly by alcohol's effects on NMDA receptors. The induction and maintenance of LTP is heavily dependent upon BDNF and NMDA receptors, and therefore alcohol exposure may also cause intellectual, memory, and cognitive deficits by altering this pathway. In addition to this, alcohol also acts as a $GABA_A$ receptor agonist. GABA-mimetic agents such as alcohol increase excitation in immature neurons, causing hyperactivation and apoptosis during development. The inhibition of NMDA receptors and the activation of $GABA_A$ receptors results in widespread apoptosis in rodent models. Taken together, alcohol exposure during development can cause apoptosis through both GABA agonism and NMDA antagonism and as a result, negatively affect neurotrophin expression, LTP, and the formation of neural circuits in the developing brain [100].

It should be noted that the severity and type of effects from alcohol exposure depend on the intensity of exposure, duration of exposure, and at what time during development the exposure takes place. Regardless of these variables, it is clear that key neurotrophins, their receptors, and downstream effectors essential for normal neuronal and synaptic development are negatively impacted by prenatal exposure to alcohol.

Summary

Neurotrophins are a small family of growth factors that have unique expression patterns and act on distinct neuronal populations at different stages in development and adulthood. Neurotrophins are essential for the developing nervous system because they regulate neuronal survival, differentiation, and connectivity. Dysregulation of neurotrophins at critical stages throughout early development results in neurodevelopmental disorders that arise from perturbations in neuronal proliferation, differentiation, synaptic function, and plasticity.

Multiple Choice Questions (* = correct answer)

1. Which of the following statements best describes caspases?
   A. Enzymes that are inactivated during apoptotic signaling, triggering cleavage of subcellular structures
   B. Organelles that cleave inactive enzymes that trigger apoptotic signaling
   C. Enzymes that are cleaved by proNGF that trigger apoptosis
   D. Enzymes that, when activated by cleavage, trigger apoptosis**
2. Under what conditions will proNGF exhibit neurotrophic activity?
   A. When TrkB and $p75^{NTR}$ are expressed
   B. When only p75 is expressed in the presence of the Lingo-1 ligand
   C. When TrkA and $p75^{NTR}$ are expressed**
   D. proNGF does not exhibit neurotrophic activity
3. Why is NT-3 critical for proper nervous system development?
   A. It is the last neurotrophin expressed during development and is critical for late prenatal plasticity
   B. It is the first neurotrophin expressed during development and promotes gliogenesis in astrocytes
   C. It is the first neurotrophin expressed during development and is involved in ENS neuron development and myelination**
   D. It is expressed at the same time as all other neurotrophins and acts in concert with them to aid in the development of both the ENS and CNS
4. The Trophic theory of development states that:
   A. Neurons and their connections are pruned during development because trophic factors are synthesized in abundance by target tissues
   B. The neurons and connections that survive during development are those that compete successfully for limited amounts of trophic factors secreted by target tissues**
   C. Neurotrophins are the only trophic factors required by developing neurons
   D. Neurons make multiple connections with their target tissues because their target tissues secrete high amounts of trophic factors during development
5. LTP is a model for:
   A. Learning and memory*
   B. Cell signaling
   C. Apoptosis
   D. Embryogenesis

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Synaptogenesis

5

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Abbreviations

ASD	autism spectrum disorder
BDNF	Brain-Derived Neurotrophic Factor
C1q	complement component 1q
C3	complement component 3
Ca^{2+}	calcium
CaMKII	calcium/calmodulin-dependent kinase II
CASK	calcium/calmodulin-dependent serine protein kinase
Cbln1	cerebellin 1 precursor protein
Cl–	chloride
CNS	central nervous system
CR3	complement receptor 3
E/I	excitatory/inhibitory
ECM	extracellular matrix
FGF	fibroblast growth factor
FMRP	Fragile X Mental Retardation Protein
GABA	gamma-aminobutyric acid
GluRδ2	glutamate receptor-like molecule δ2
LGN	Lateral Geniculate Nucleus
MAGUK	Membrane-associated Guanylate Kinase
MEF2c	myocyte enhancer factor 2c
MEGF10	multiple EGF-like domains 10
mRNA	messenger RNA
Munc13	Mammalian Homolog of Unc13
MuSK	Muscle specific kinase
Na+	sodium
NCAM	neural cell adhesion molecule
NMDA	N-methyl-D-aspartate
NMJ	neuromuscular junction
Pcdh10	protocadherin 10
PSD	postsynaptic density
PSD-95	postsynaptic density protein-95
RGCs	retinal ganglion cells
RIM-BPs	RIM-Binding Proteins
RIMs	Rab3A-Interacting Molecules
SM	Sec1/Munc18-like
SNARE	SNAP receptor
TREM2	triggering receptor expressed on myeloid cells 2
TSP2	thrombospondin-2

Learning Objectives

Upon completion of this chapter, you should be able to:

1. Describe the types and structure of synapses, and the major molecular components at the pre- and postsynaptic compartments.
2. Understand the biological process of synapse formation including pre- and postsynaptic structure initial contact, recognition, differentiation, and maturation.
3. Explain how glial cells are involved in synapse formation.
4. Understand the general process of synapse remodeling and elimination and the implications of synapse dysregulation in neurological diseases.

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Highlights

- The synapse is the most fundamental structure in the brain responsible for neuronal communication and brain function.
- During synapse formation, the axon terminal and the dendritic spine make contact and recognize each other *via* adhesion molecules, leading to bidirectional signaling and differentiation of the presynaptic terminal (such as machineries for neurotransmitter release) and the postsynaptic spine (such as receptor insertion into the postsynaptic membrane).
- Synapses are dynamically regulated during brain development and dysregulation of the process plays an important role in neurological disorders.

Overview of the Structure of Synapses in the Central Nervous System

To lay the foundation for understanding synaptogenesis, begin by considering the mature synapse. The fundamental mechanism of synaptic transmission is the same at all chemical synapses. An action potential initiated near the presynaptic neuronal cell body propagates along the long, thin projection called the axon to the specialized nerve terminal where it triggers calcium (Ca^{2+}) influx through voltage-gated Ca^{2+} channels. This induces vesicle-mediated release of neurotransmitter from the presynaptic terminal into the synaptic cleft. The released transmitter then binds to receptors on the postsynaptic membrane, usually on dense, branched projections of the postsynaptic cell called dendrites. The postsynaptic receptors then transduce the signal into a change in voltage across the membrane and/or activation of signaling cascades within the postsynaptic cell. However, the molecular composition of pre- and postsynaptic specializations differs among synapses in important ways. For example, in the mature central nervous system (CNS), synapses that use the transmitter glutamate mediate most fast excitatory neurotransmission, while gamma-aminobutyric acid (GABA) is the primary neurotransmitter for inhibitory transmission. Hence, while some aspects of chemical synapses are universal, others differ among synapse types.

In addition to chemical synapses, the brain contains numerous electrical synapses which permit direct propagation of electrical current from one cell to another through precisely aligned pores called gap junctions. Electrical synapses play important roles in nervous system development and, like chemical synapses, their dysfunction has been associated with neurodevelopmental disease [1]. However, this chapter will focus on chemical synapses as these are thought to underlie most complex information processing in the CNS, including learning and memory, and have been implicated in a wide range of developmental disorders affecting cognition.

The Presynaptic Compartment

The most salient features at presynaptic terminals are the large numbers of neurotransmitter-packed vesicles present there, some of which are docked at the membrane in preparation for release upon calcium influx following an action potential. All presynaptic terminals contain synaptic vesicles packed with small molecule neurotransmitters such as glutamate, GABA, glycine, acetylcholine, norepinephrine, dopamine, or serotonin. Neurons generally produce only one of these transmitters, although co-release has also been reported, particularly in the developing nervous system [2]. Within the presynaptic terminal, small molecule neurotransmitters are generated from precursors by specific enzymes in the cytoplasm and packaged into vesicles by transmitter-specific vesicular transporters. Thus, the complement of presynaptic vesicular transporters and enzymes varies among neurons along with the particular neurotransmitter(s) they release.

Nerve terminals may also contain another type of vesicle, named large dense-core vesicles because of their larger size and dark appearance electron micrographs. Large dense-core vesicles carry modulatory neuropeptides such as oxytocin, and/or neurotrophic factors, such as brain-derived neurotrophic factor (BDNF). Neuropeptides and neurotrophic factors are differentially expressed between neurons and are often co-released with small molecule neurotransmitters. However, in contrast to synaptic vesicles, dense core vesicles are present throughout the cell, and their release is not as tightly coupled to activity. Although dense core vesicles do not directly mediate synaptic transmission, neurotrophic factors and neuropeptides play important modulatory roles in synaptic transmission and regulate synapse formation.

In addition to synaptic vesicles, presynaptic terminals contain an elaborate molecular infrastructure to permit precisely timed, activity-dependent vesicle release, as well as recycling of vesicles by endocytosis. Vesicle release occurs at regions called active zones, which contain machinery evolved to allow fusion of the lipid bilayer of the vesicle with that of the nerve terminal. The active zone is evident in electron micrographs as an electron-dense submembrane structure at which vesicle fusion occurs [3]. While the particular neurotransmitters employed varies among synapses, the molecules of the active zone and vesicle fusion apparatus are conserved across synapse types. At the core of the vesicle fusion apparatus is the SNAP Receptor (SNARE) complex, which consists of the plasma membrane-anchored proteins Syntaxin and SNAP-25 and the vesicle-anchored protein

synaptobrevin [4]. This complex interacts with Sec1/Munc18-like (SM) proteins, which play important roles in SNARE complex formation, and with synaptotagmin, a Ca^{2+}-sensitive synaptic vesicle protein that mediates Ca^{2+}-dependent fusion. Upon Ca^{2+} influx, synaptotagmin triggers a conformational change in the SNARE complex resulting in a zippering action that drives the vesicular and plasma membrane together, causing membrane fusion and release of vesicle contents into the synaptic cleft [4]. The active zone plays a crucial role in ensuring rapid activity-dependent secretion. Active zone scaffolding proteins Rab3A-interacting molecules (RIMs), RIM-binding proteins (RIM-BPs), and Mammalian Homolog of Unc13 (Munc13) function together to create a microdomain in which vesicle release can be rapidly and transiently induced by local Ca^{2+} influx [3, 5]. While Munc13 primes vesicles for fusion through interactions with the Sec1/Munc18-like (SM) and SNARE fusion machinery, RIMs and RIM-BPs link vesicles with voltage-gated Ca^{2+} channels through protein–protein interactions. This arrangement ensures the tight coupling of vesicle fusion to local increases in Ca^{2+}.

Other important active zone adaptor proteins control the localization of vesicle release to the synapse by linking the vesicle release machinery with trans-synaptic adhesion molecules that maintain the alignment of the pre- and postsynaptic specializations. For example, calcium/calmodulin-dependent serine protein kinase (CASK), and Mints interact with components of the vesicle release machinery on the one hand and with trans-synaptic cell adhesion molecules on the other [3]. The presynaptic adhesion proteins expressed differ between cell types [6], and the importance of this variability for development and developmental brain disease will be discussed in later sections. Clearly, assembly of the presynaptic specialization involves intricate and precise interactions among an enormous number of proteins (Fig. 5.1).

The Postsynaptic Compartment

Relative to the presynaptic specialization, the postsynaptic specialization shows greater divergence among synapses (Fig. 5.2). Important insight into postsynaptic differentiation has come from comparison of postsynaptic compartments opposed to excitatory neurons, most of which are glutamatergic, and inhibitory neurons, most of which are GABA- or glycinergic.

At the molecular level, the most obvious postsynaptic difference between excitatory and inhibitory synapses is in the receptors present in the membrane. At excitatory glutamatergic synapses, the postsynaptic membrane contains ionotropic receptors that trigger a depolarizing influx of sodium (Na+) upon glutamate binding. In contrast, at inhibitory synapses, the postsynaptic membrane contains ionotropic receptors that trigger a hyperpolarizing influx of chloride (Cl−) ions when activated by GABA or glycine binding. In addition to ionotropic receptors, synaptic membranes also contain metabotropic receptors for specific neurotransmitters, which mediate slow synaptic transmission by activating intracellular signaling cascades important for homeostasis and synaptic plasticity.

At both excitatory and inhibitory synapses, postsynaptic receptors are restricted from diffusing away from the synapse by their association with an elaborate submembrane system of interacting scaffolding proteins, cytoskeletal elements, and transmembrane proteins. This postsynaptic scaffold also contains important intracellular signaling molecules that mediate short- and long-term synaptic plasticity. Indeed, the scaffold itself is not rigid and fixed but fluid and dynamic. At glutamatergic, excitatory synapses the postsynaptic scaffold is known as the postsynaptic density (PSD) and is visible by electron microscopy as a prominent electron-dense region opposite the active zone, within a specialized protrusion from the dendritic shaft called a dendritic spine. Like the postsynaptic scaffold, the dendritic spine is highly dynamic. In contrast, inhibitory synapses are located directly on the dendritic shaft, and the postsynaptic scaffold structure is much thinner resulting in a more symmetrical appearance [7]. Indeed, synapses have long been classified as asymmetrical (generally corresponding to excitatory synapses) or symmetrical (generally corresponding to inhibitory synapses) based on electron micrographs. Interestingly, these ultrastructural differences in the postsynaptic specialization are accompanied by presynaptic differences in vesicle shape, with a greater proportion of ellipsoid, as opposed to spherical, vesicles at inhibitory synapses, although the significance of this difference in vesicle shape is not clear.

Membrane-associated guanylate kinase (MAGUK) scaffolding proteins serve as key organizers of the excitatory PSD through diverse concurrent protein–protein interactions mediated by their PDZ domains and other protein–protein interaction domains. The MAGUK postsynaptic density protein-95 (PSD-95), beneath the postsynaptic plasma membrane, is thought to play a particularly important organizing role by interacting with plasma membrane proteins including N-methyl-D-aspartate (NMDA)-type glutamate receptors [8], AMPA receptor regulatory proteins such as stargazin [9], the trans-synaptic adhesion protein neuroligin [10], and potassium channels [11], while simultaneously interacting with the cytoskeleton [12] to anchor these components within the PSD. Further illustrating its central role in the elaborate interconnectivity of the excitatory PSD, PSD-95 also interacts with the scaffolding proteins guanylate kinase-associated protein (GKAP), which binds to Shank [13]. Shank in turn interacts with another prominent scaffolding protein, Homer1, which binds metabotropic glutamate recep-

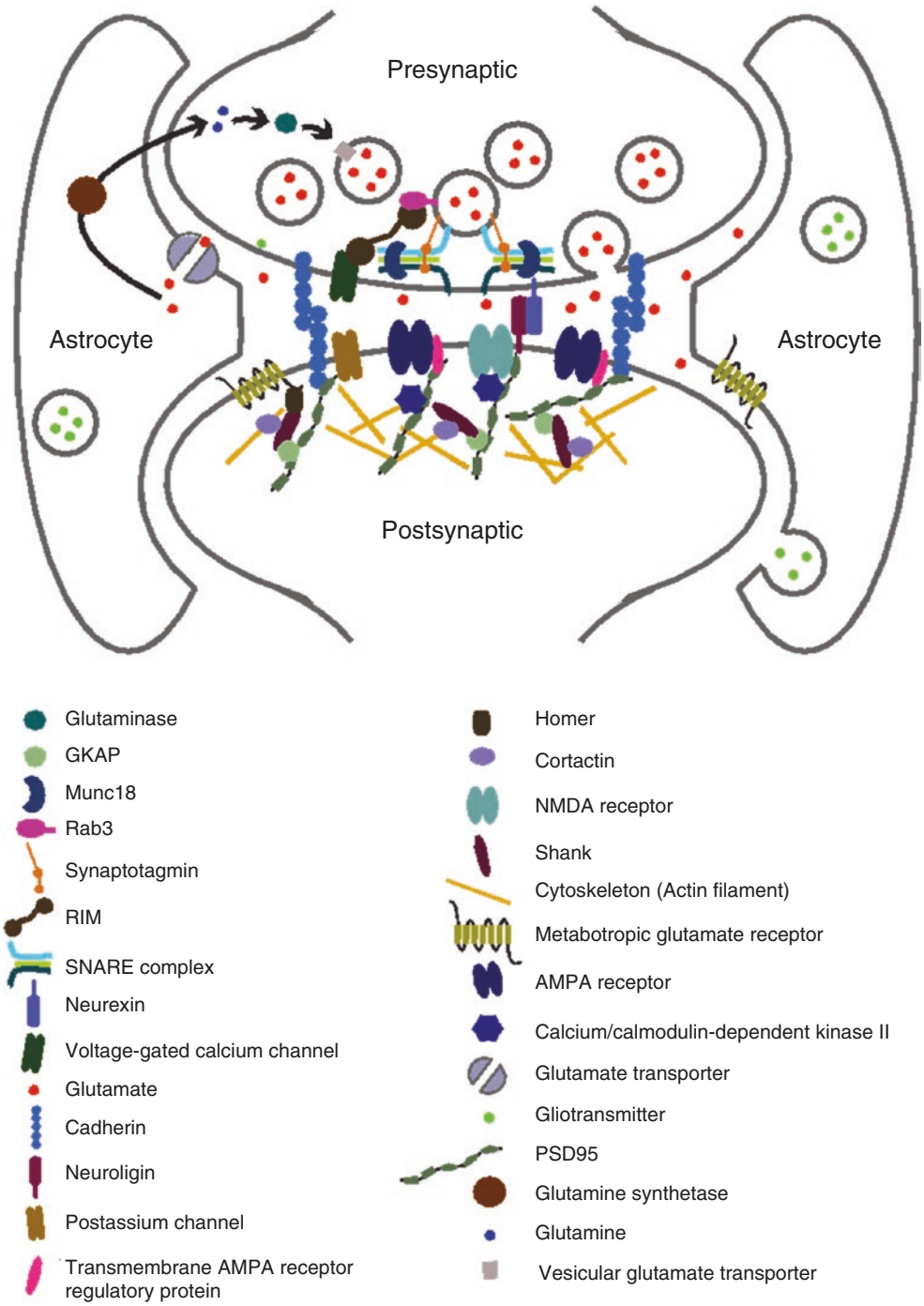

Fig. 5.1 Molecular composition of an excitatory synapse. Synapse formation involves the establishment of an intricate meshwork of presynaptic, trans-synaptic, and postsynaptic interacting proteins. The properties of the vesicle fusion machinery and active zone constituents ensure that neurotransmitter release is tightly coupled to calcium influx. Trans-synaptic adhesion proteins bridge the pre-and postsynaptic apparatus and maintain alignment of the active zone and postsynaptic density. The scaffolding proteins of the postsynaptic density facilitate signal transduction by interacting with postsynaptic receptors, signaling proteins, and cytoskeletal proteins

tors to facilitate metabotropic signal transduction at the PSD (Fig. 5.1). The postsynaptic specialization appears to be organized according to similar principles at inhibitory synapses, but distinct molecular players fill the key organizing roles. Instead of PSD-95, the scaffolding protein Gephyrin occupies the central scaffolding niche at inhibitory synapses, binding to receptors for glycine and GABA as well as to cytoskeletal components and to Collybistin, a lipid-binding postsynaptic scaffolding protein (Fig. 5.2) [14]. Gephyrin plays a critical role in the clustering of receptors at inhibitory postsynaptic sites [15].

Finally, as mentioned earlier, trans-synaptic adhesion proteins are present on both the postsynaptic and presynaptic membrane, where they interact across the synaptic cleft through homophilic or heterophilic binding and play important roles in synapse formation and differentiation (Figs. 5.1

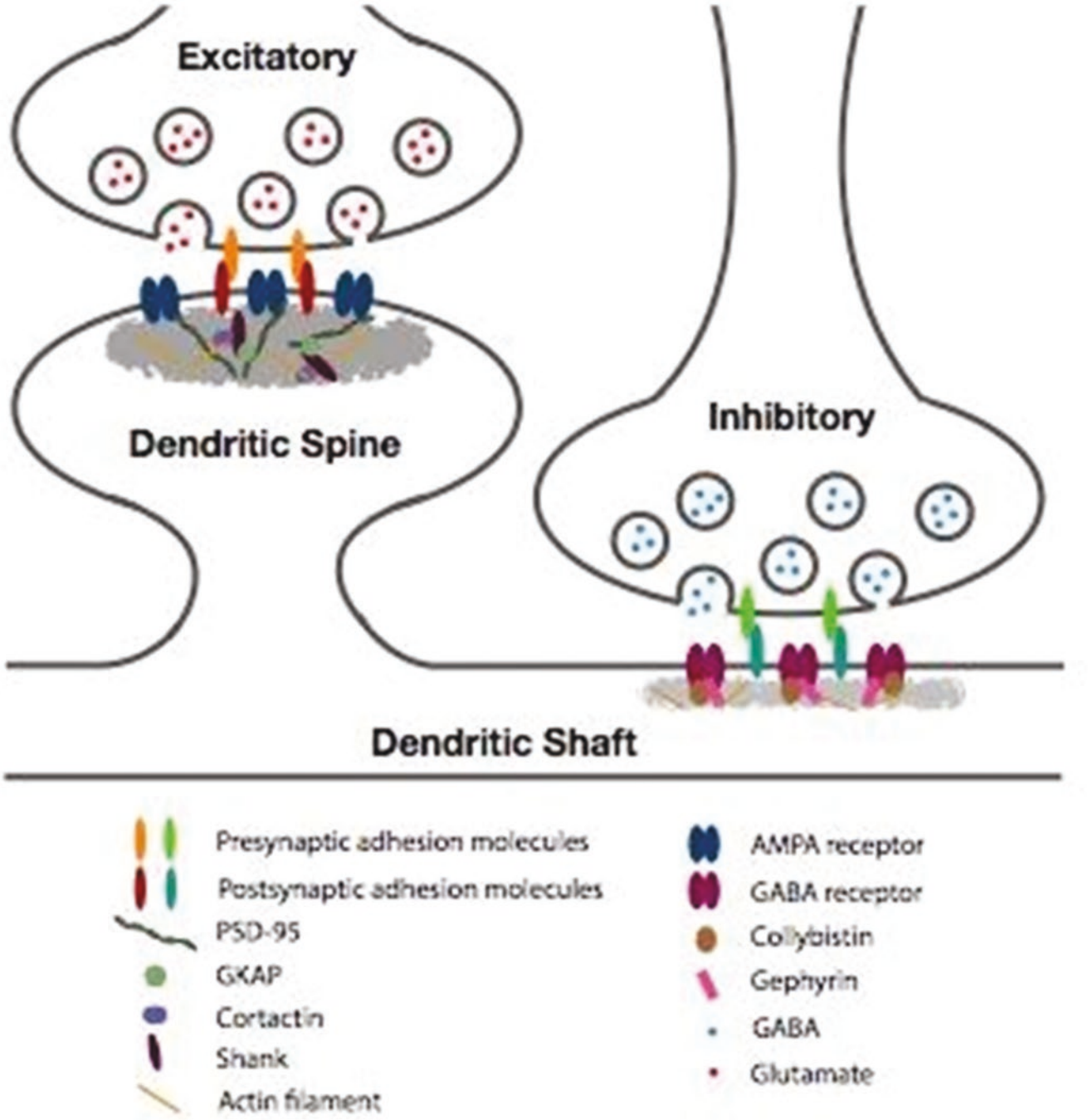

Fig. 5.2 Comparison of excitatory and inhibitory synapses. Excitatory and inhibitory synapses differ in molecular constituents, including postsynaptic receptors and scaffolding proteins, as well as trans-synaptic adhesion proteins

and 5.2). There are a great number and diversity of adhesion proteins present in postsynaptic membranes, including neuroligins, cadherins, latrophilin-2, synaptic cell adhesion molecules (synCAMs), and the Eph receptor, among others [16, 17]. Most are transmembrane proteins with many distinct isoforms and splice variants, and different neuronal subtypes express different adhesion proteins, particularly on the postsynaptic side. This diversity hints at the elaborate and precise interplay of molecular interactions driving the formation of appropriate synapses and circuits during development.

Astrocytes: A Third Compartment at the Synapse

The role of glia in synaptic function and development has been relatively neglected until recently. In addition to the pre- and postsynaptic compartments, many CNS synapses include a third compartment consisting of a process emanating from a glial cell called an astrocyte, which may ensheathe the synapse and participate in synaptic regulation [18, 19]. Through these processes, astrocytes can mediate neurotransmitter reuptake and recycling back to neurons [20] and regulate ion trafficking [21–23]. Astrocytes can express receptors to sense neurotransmitters, and they can also synthesize and release gliotransmitters to affect neuronal function [24]. There is evidence of bidirectional chemical signaling between astrocytes and neurons, which may affect synaptic transmission [19]. In addition to astrocytes, other glial cell types also secrete transmitters that affect neuronal synapses, and in some brain regions, synapse-like direct contact may be established between neurons and specific glial cells for rapid communication [25, 26]. In later sections, we will discuss the important roles that astrocytes and other glia play in the development of CNS connectivity.

Synaptogenesis

The establishment of mature synaptic connectivity can be divided into three phases: initial contact, synapse assembly, and synapse elimination. With regard to the last phase, it is well established that the brain makes many more connections than persist into the adult. Elimination of inappropriate synapses is key to proper function of the visual system, neuromuscular junction, and many other parts of the nervous system. Synapse formation and elimination continue throughout life in certain parts of the nervous system [27], and are thought to be important for brain functions including learning and memory; however, here we will focus only on the initial contact, synapse assembly, and synapse elimination that occur during development.

Early insights into synaptogenesis came from studies of the peripheral synapses between motor neurons and skeletal muscle fibers, known as the neuromuscular junction (NMJ). Due to its simplicity, accessibility, and large size, the NMJ has been a highly valuable model system in the study of synaptogenesis. Although this model will not be discussed in detail here, it will be referenced occasionally for context, as many of the principles currently understood to govern synapse formation in the CNS were originally drawn from this simple model system.

Initial Contact

To explore the environment and contact potential partners, neurons extend thin, motile protrusions called filopodia, which are supported internally by dynamic actin filaments [28]. Filopodia can emerge from growth cones at the end of extending axons [29] or dendrites [30] or directly from dendrite shafts [31, 32], and in the latter circumstance, the filopodia have been proposed to be the precursor of dendritic spines [28] (Fig. 5.3).

Disruption of filopodia/spine dynamics may play a role in certain brain developmental disorders, which are often accompanied by alterations in dendritic spine density and/or morphology and are sometimes linked to changes in actin dynamics. Brains from patients with fragile X syndrome, the most common monogenic cause of autism, have been reported to have a disproportionately high number of immature-looking spines [33]. Furthermore, this aberrant spine morphology was ameliorated by manipulating actin-remodeling proteins including profilin 1, Rac1, and group I p21-activated kinases [34]. Similarly, manipulations of cofilin and Rac1 also partially rescued behavioral and functional phenotypes in a Shank3 loss-of-function mouse model mimicking human Phelan-McDermid syndrome, a disorder involving developmental delay and autistic behavior [35]. Consistent with altered filopodia and spine dynamics in autism, a number of the autism-linked genes identified in human genetic studies are involved in regulation of actin polymerization, such as alpha-actinin, cortactin-binding protein 2, and several Rho GTPase-activating proteins, among others [36]. Together these observations suggest that certain neurodevelopmental diseases may result in part from defects in filopodia and spine dynamics.

Synapse Assembly

Of the initial contacts formed between neurons, most are retracted, while a very few stabilize into mature synapses (Fig. 5.3) [31, 37, 38]. Cell surface adhesion molecules appear to play an important role in mediating mutual recognition and promoting the assembly of appropriate synapses between suitable partners. Many neuronal adhesion molecules, when overexpressed in non-neuronal cells, have been found to induce formation of pre- or postsynaptic specializations in co-cultured neurons, and a number of these have also been shown to impair synapse formation or alter synapse properties when knocked out in mice. These include neurexin [39], neuroligin [40], latrophilin, cadherin, SynCAM [41], neural cell adhesion molecule (NCAM), ephrin, and the Eph receptor among many others. The number of neuronal adhe-

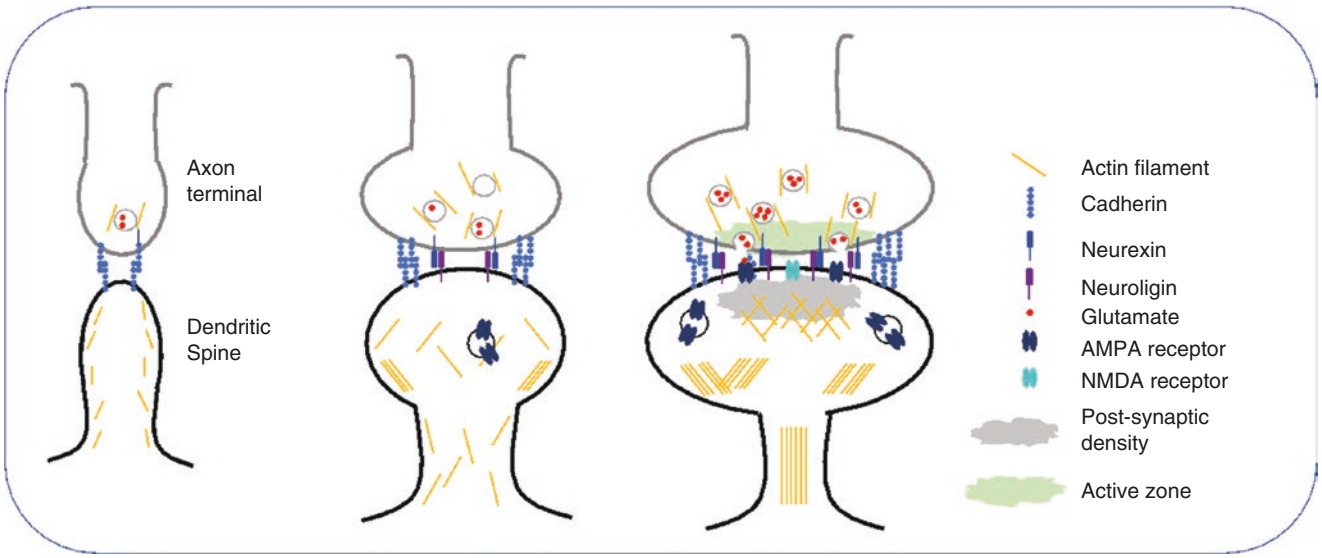

Fig. 5.3 Formation and maturation of synapses. Adhesion molecules facilitate the initial recognition and contact of the axon terminal and the dendritic spine. The pre- and postsynaptic structures will then differentiate with the recruitment of functional components including neurotransmitter vesicles, receptors, and postsynaptic scaffolding molecules

sion proteins implicated in synaptogenesis has been further expanded by the identification of closely related isoforms and variants. For example, the cadherin superfamily includes a great diversity of isoforms due to the existence of multiple genes in the superfamily, use of alternative promoters, and especially extensive alternative splicing [42]. Similarly, there are three gene-encoding isoforms of the presynaptic transmembrane protein neurexin in mammals, [43, 44] and five genes encoding its postsynaptic-binding partner neuroligin have been identified in humans [45–47], each of which encodes a variety of alternative transcripts and splice variants.

Most synaptic adhesion proteins are transmembrane proteins and, in addition to interacting with one another extracellularly in isoform-specific ways, they also engage in isoform-specific interactions with intracellular proteins. Thus, diverse adhesion proteins, combined in distinctive permutations at different synapse types, provide a compelling mechanism for mutual recognition, as well as for translating that recognition into intracellular changes. For example, the extracellular domains of different cadherin and protocadherin isoforms and variants have different binding affinities, binding in a homophilic manner to the same isoform and/or in a heterophilic manner to distinct isoforms, and are differentially expressed among synapse types [42]. This may provide a mechanism for cell–cell recognition [48]. Similarly, while neuroligin 1 localizes to excitatory synapses [49], interacting intracellularly with PSD-95 [10], neuroligin 2 is present at inhibitory synapses [39, 50] and interacts with gephyrin and collybistin [51, 52]. Supporting a synapse type-specific role, manipulations of neuroligin 1 alter the molecular and electrophysiological properties of excitatory but not inhibitory synapses in culture and in vivo, while manipulations of neuroligin 2 alter the properties of inhibitory but not excitatory synapses [53].

Important insights into synapse assembly have come from the study of the NMJ. NMJ formation and regeneration depends on key molecules within the extracellular matrix (ECM) of the synaptic cleft [54, 55] and accumulating evidence suggests that ECM molecules also play a key role in initiating synapse assembly in the CNS [56, 57]. Of special interest is cerebellin 1 precursor protein (Cbln1), a glycoprotein secreted by cerebellar granule cells that is required for formation of cerebellar Purkinje cell synapses in mice. Cbln1 appears to act as a bridge between cell surface proteins on pre- and postsynaptic cells, binding neurexin pre-synaptically and interacting post-synaptically with glutamate receptor-like molecule $\delta 2$ (GluR$\delta 2$), a cerebellar Purkinje cell-specific degenerate glutamate receptor subunit [57]. Thus, in this case, a tripartite trans-synaptic complex is required for synapse formation.

At the NMJ, synapse formation involves activation of a transmembrane receptor tyrosine kinase on the postsynaptic muscle cell, called muscle specific kinase (MuSK), by the extracellular matrix proteoglycan agrin, secreted from the presynaptic motor neuron [58]. However, unlike the NMJ, the putative trans-synaptic organizers at central synapses appear to lack catalytic activity, suggesting any effects on intracellular signaling must be indirect. It is likely that trans-synaptic adhesion proteins affect intracellular signaling through direct or indirect interactions with intracellular enzymes. Indeed, neurexins interact with the MAGUK-family kinase CASK, which can phosphorylate itself, neurexin and possibly other targets as well [59, 60].

Once an appropriate target cell has been identified, the pre- and postsynaptic specializations form rapidly from components that are, for the most part, already present in the cells involved (Fig. 5.3). In cultured neurons that have not yet begun to form synapses, NMDA and AMPA receptor subunits are present in dendrites where they form clusters [61–63], and synaptic vesicle proteins synapsin-1 and synaptophysin are present in the distal axons [64]. Active zone constituents and synaptic vesicle-associated proteins are present in vesicles within the axon and are delivered to the presynaptic site during synapse maturation [38, 65, 66]. Similarly, in the early stages of synaptogenesis, non-synaptic complexes of postsynaptic scaffolding proteins and transsynaptic adhesion proteins form in cultured neurons, supporting the idea that the components of the PSD may be partially pre-assembled to permit efficient synapse formation upon cell–cell contact [67].

A striking number of autism risk genes identified in genome-wide association studies encode proteins involved in synapse specification and assembly. The postsynaptic scaffolding proteins PSD-95, Homer and Gephyrin have been identified as autism-associated genes [68]. Cadherins, neuroligins, neurexins and other adhesion molecules are also among the identified autism-associated genes, as are cerebellins [68]. This strongly suggests impairment in synapse formation, specification or plasticity in a subset of individuals with autism. Synapse assembly roles have also been identified for many of the genes responsible for monogenic neurodevelopmental disorders. As mentioned above, disruption of the gene encoding the postsynaptic scaffolding protein Shank3 leads to Phelan-McDermid syndrome [69]. Interestingly, Shank3 gene duplication is also associated with neurodevelopmental diseases including autism and schizophrenia, and overexpression of Shank3 in mice results in an altered behavioral phenotype, suggesting this synaptic protein must be precisely regulated [70]. Similarly, loss-of-function mutations in the presynaptic scaffolding protein CASK lead to developmental disorders involving autistic behavior, developmental delay, epilepsy, hypotonia, and other physical and sensory symptoms [71, 72]. In addition, investigation into the mechanisms of Fragile X syndrome have identified the Fragile X Mental Retardation Protein (FMRP) as a messenger RNA (mRNA)-binding protein that regulates local dendritic translation of a number of synaptic proteins including PSD-95 [73]. Altered synapse assembly thus likely contributes to the behavioral alterations and aberrant synaptic morphology observed in certain monogenic and polygenic neurodevelopmental disorders.

Neuronal and Glial Secreted Molecules in Synaptogenesis

Although direct cell–cell contact is required for assembly of the mature synapse, secreted molecules also play important roles in synapse formation and maturation. As described above, neuronal secretion of ECM proteins, like cerebellin-1, plays an important role in synapse assembly by forming a bridge between pre- and postsynaptic cell surface proteins. Other well-characterized neuronal-secreted ECM proteins include the pentraxins, which interact with AMPA receptors and stimulate their aggregation [74]. In addition, a number of freely diffusing secreted proteins can modulate synaptic connectivity, including BDNF, Wnts, and fibroblast growth factor (FGF). Exogenous BDNF treatment increased measures of synapse formation in both in vitro [75] and in vivo model systems [76], and experiments with genetic mouse models have confirmed that BDNF is required for normal synaptic connectivity in certain brain regions [77]. A portion of BDNF expression and release is regulated by neuronal activity, and disrupting activity-related BDNF synthesis in mice alters inhibitory synapse development, suggesting a role in regulating the excitatory/inhibitory (E/I) balance [78]. Similar roles in regulating synapse formation and E/I balance have been reported for Wnt-7a and FGF [79–82]. Interestingly, distinct FGF isoforms secreted from dendrites have been found to stimulate the formation of excitatory or inhibitory synaptic inputs [81, 82]. Thus, neuronal secreted molecules have essential roles in mediating and modulating synaptic development and establishing the appropriate balance between excitatory and inhibitory connectivity.

In addition to the secreted factors described above, the axons of developing neurons secrete neurotransmitter even before cell–cell contact is made, and experiments using local release of exogenous neurotransmitters have revealed that glutamate is sufficient to induce the formation of dendritic spine-like structures [83], while GABA is sufficient to induce assembly of inhibitory and excitatory postsynaptic structures in cultured neurons [84]. These findings suggest that release of neurotransmitter from immature neurons may facilitate in the formation of excitatory and inhibitory synapses, although formation of synapse-like structures can also occur in the absence of neurotransmitter release [85].

Growing evidence points to an important role for glial secreted proteins in synaptogenesis. Mixed neuron-glia cultures are known to form significantly more synapses than pure neuronal cultures [86], and a number of astrocyte-secreted factors, such as the ECM proteins thrombospondins, glypicans, and hevin have been shown to positively

modulate excitatory synapse formation *in vitro* and *in vivo* [87–90]. One of the best-known synaptogenic gliotransmitters is thrombospondin 2 (TSP2). Upon release from astrocytes, TSP2 binds to neuronal Gabapentin receptor α2δ-1, a known anti-epileptic drug target, to initiate Rac1-dependent formation of excitatory synapses [87, 91, 92]. Interestingly, the new synapses induced by treatment with TSP2 are non-functional; while they are presynaptically active, there is a lack of neurotransmitter receptors at the postsynaptic site [87]. Hevin similarly was found to induce formation of postsynaptically silent synapses through a mechanism involving interaction with presynaptic neurexins and postsynaptic neuroligins [93]. Subsequent studies identified glypicans 4 and 6 as the first astrocyte-secreted molecules capable of inducing formation of fully functional excitatory synapses [90]. Glypican 4 has since been shown to induce AMPA receptor clustering in part by stimulating presynaptic release of the synaptic organizer pentraxin 1 [89]. In contrast to these positive regulators, another astrocyte-secreted factor, SPARC, negatively regulates excitatory synapse formation by antagonizing hevin [88]. Microglia also secrete synaptogenic molecules, including BDNF [94], and may contribute to synapse formation during development. It has also been proposed that glia may regulate synapse formation through a contact-dependent mechanism, although this has not been investigated in depth [95].

Dysregulation of secreted synaptogenic signals may be a contributing factor in certain brain developmental disorders. A number of autism risk genes encode Wnt signaling pathway regulators and components, including several Wnt isoforms [96–98], and systems biology studies have identified the Wnt signaling pathway as part of a core network of genes dysregulated in autism spectrum disorder (ASD) [99]. Notably, both Wnt and FGF signaling are modulated by glypican gliotransmitters [100]. Further supporting a role for secreted signaling in developmental diseases of the CNS, synaptic abnormalities in the brains of individuals with Downs syndrome have been attributed to a deficit in thrombospondin-1 [101], and genetic variants of thrombospondin-1 have been associated with ASD [102]. ASD is associated with increased peripheral BDNF levels [103], while decreased levels are observed in schizophrenia [104]. Interestingly, investigation into the genetic basis of Rett syndrome, a neurological disorder involving developmental delay and other behavioral and physical symptoms, led to the discovery of a signaling cascade in which calcium/calmodulin-dependent kinase II (CaMKII)-mediated phosphorylation of MeCP2, the gene mutated in Rett syndrome, leads to increased BDNF expression and spine formation [105].

Synapse Remodeling and Elimination

Although it may seem counterintuitive, synapse elimination plays a major role in the establishment of mature synaptic circuitry in the CNS. In humans, abundant synapse formation in the first two postnatal years is followed by a prolonged synapse elimination phase lasting approximately 15 to 20 years [106]. Findings from diverse model systems point to neuronal activity as an important driver of this synapse elimination.

The visual system has proved a valuable model for understanding activity-dependent synapse elimination in part because of the ease with which activity of retinal projections from each eye can be independently manipulated. Classic studies by Hubel and Wiesel revealed that the mature connectivity of the visual cortex in cats and primates is determined by the animal's visual experience during a critical period in its development [107]. Eliminating visual input to one eye during this critical period permanently decreased that eye's representation in the visual cortex, and the other eye's representation was increased accordingly. This suggested that synaptic connectivity is not hard-wired but subject to remodeling based on experience, and early, activity-dependent competition between the eyes drives the establishment of mature visual circuitry.

Further insight into the mechanisms of activity-dependent synapse refinement in the mammalian visual system has come, in part, from studying the synaptic connections of the retinal ganglion cells (RGCs) onto relay neurons in the lateral geniculate nucleus (LGN) of the thalamus (Fig. 5.4). Initially, RGC projections from both eyes form abundant, indiscriminate synaptic contacts with relay neurons of the LGN. Then a period of synapse formation and elimination results in segregation of inputs from the two eyes into separate territories within the LGN [108–111]. Much of this synapse reorganization takes place before eye-opening but coincides with waves of spontaneous synchronized neuronal activity in the retina, and disrupting these waves of activity in one eye results in a reduction in LGN innervation by that eye and an increase in innervation by the other eye [110, 112]. This implies that activity-based competition drives the segregation of retinogeniculate synapses from the two eyes. Like activity-dependent plasticity mechanism proposed to underlie memory, often paraphrased as "cells that fire together wire together," activity-dependent elaboration and elimination of retinogeniculate synapses is thought to reflect the elaboration of contacts between neurons that fire in synchrony, and elimination of contacts between neurons whose activity is out of sync.

During the development of peripheral synapses between motor neurons and skeletal muscle fibers, there is a similar

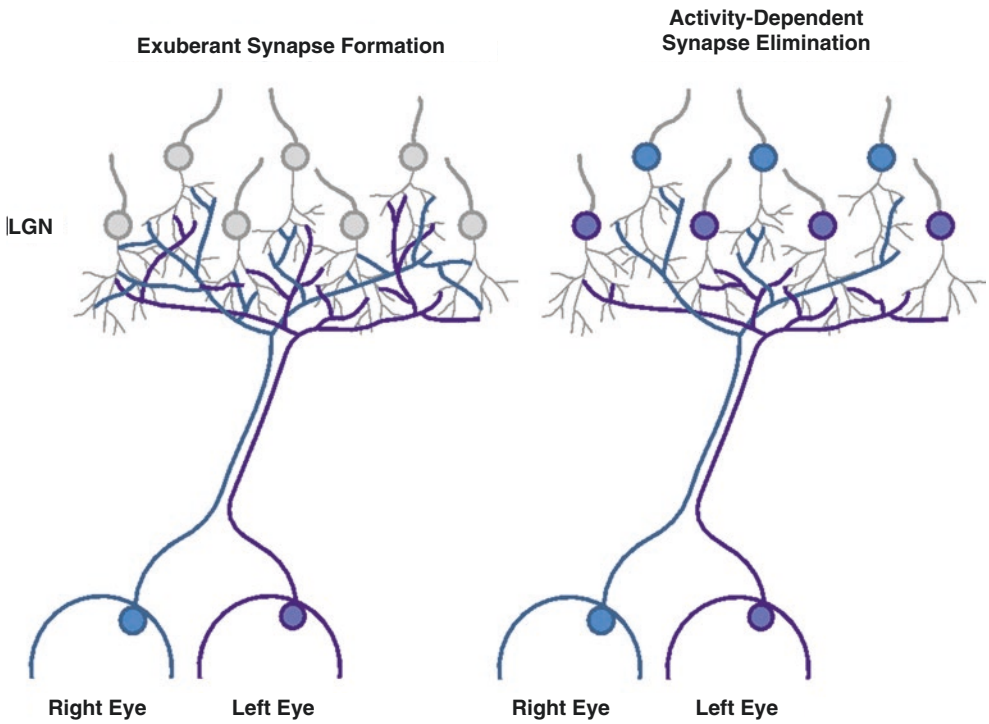

Fig. 5.4 Activity-dependent synapse elimination. Retinal projections from the two eyes initially form abundant and indiscriminate synapses onto neurons of the lateral geniculate nucleus (LGN). Subsequent activity-dependent synapse elimination results in the formation of separate right and left eye innervation territories

initial hyperconnectivity followed by selective activity-dependent elimination of inputs, with the end-result that each twitch muscle fiber receives input from only one motor neuron [58]. This synapse elimination has been shown to involve neurotrophins, particularly the activity-regulated neurotrophin BDNF [113, 114], and growing evidence points to a similar role for BDNF isoforms in synaptic pruning in the CNS [115, 116]. A portion of BDNF mRNA is trafficked to dendrites and synthesized locally in an activity-dependent manner, and mice lacking local dendritic translation of BDNF exhibit reduced developmental synaptic pruning in the hippocampus and visual cortex, as well as aberrant ocular dominance plasticity [117, 118]. Understanding the role of BDNF in synapse elimination is complicated by the fact that pro-BDNF, the precursor from which BDNF is produced by proteolytic cleavage, acts as a neurotransmitter in its own right. In fact, pro-BDNF and the pro-domain cleaved off during the formation of mature BDNF have both been implicated in synapse elimination in the CNS [115, 116, 119]. Interestingly, mature BDNF and pro-BDNF seem to act in opposition to one another, raising the possibility that they may serve as reward and punishment signals to coordinately regulate synapse stabilization and elimination [113]. This would be a more nuanced twist on the classic "neurotrophic model" for synaptic refinement, which proposes that synapse elimination results from competition among synaptic inputs for target-derived neurotrophic factors [120, 121].

Although the molecular mechanisms driving synapse elimination are not well understood, a number of autism-related genes have been implicated in this process, including the transcription factor myocyte enhancer factor 2c (MEF2c), the RNA-binding protein FMRP, and protocadherin 10 (Pcdh10) [122]. Increased spine density has been reported in postmortem brain tissue from autism patients, and evidence from a mouse model suggests this could be due in part to a deficit in postnatal synapse elimination [123, 124]. In contrast, a dramatic decrease in spine density has been observed in the prefrontal cortex in schizophrenia, possibly reflecting an increase in spine elimination [125–127]. It seems likely, therefore, that altered synapse elimination contributes to the behavioral phenotypes in some patients with autism and other neurodevelopmental abnormalities.

Role of Glia in Synapse Elimination

Work on retinogeniculate pathway development has identified microglia as important mediators of activity-dependent synapse elimination. High-resolution imaging techniques have revealed phagocytosed synaptic components within microglia during the period of retinogeniculate synapse elimination in mice [128, 129] and have demonstrated that silencing of projections from one eye results in selective microglial engulfment of inputs from

that eye, matching the pattern of activity-dependent synapse elimination [129]. How microglia identify synapses destined for elimination has not been fully resolved, but deficits in segregation of retinogeniculate projections in knockout mice lacking components of the classical complement system, complement component 1q (C1q), complement component 3 (C3), or the complement receptor 3 (CR3), suggest that complement proteins may mediate the targeting of undesired synapses for phagocytosis, similar to their role in the immune system [129, 130]. The spatial and temporal expression patterns of C1q, C3, and C3R are indeed consistent with recognition and elimination of C1q- and C3-tagged synapses by C3R-expressing microglia during the period of synaptic refinement in the LGN [129, 130]. Other mechanisms may also contribute to microglia-mediated synaptic phagocytosis including the major histocompatibility complex [131], pentraxins [132], and signaling through triggering receptor expressed on myeloid cells 2 (TREM2) [133] or the fractalkine receptor CX3CR1 [134]. Astrocytes may contribute to the targeting of microglia to synapses by stimulating expression of complement proteins [130] and may also contribute directly to synapse elimination through a mechanism that depends on phagocytic MERTK and multiple EGF-like domains 10 (MEGF10) receptors [135].

The recent advances in our understanding of the role of glia in synapse formation and elimination have stimulated interest in the potential role of glia in neurodevelopmental disease. Quantification of microglia in several cortical regions has pointed to an increase in microglial numbers and activation in the brains of individuals with ASD [136]. Microglia-mediated synapse elimination within the retinogeniculate circuitry was also reported to be overactive in a mouse model of established Rett syndrome, although this may be a consequence of the loss of MeCP2 in non-microglial cells [137]. One study reported dramatic recovery in MeCP2 knockout mice following immunodepletion and bone marrow transplant from wild-type mice to replace the endogenous microglia with wild-type microglia, although the interpretation of this finding is controversial [138]. Aberrant, elevated complement activity has also been detected in serum from schizophrenia patients [139], and has been hypothesized to drive excessive synaptic pruning [140]. Complement pathway components and regulators have also been identified as some of the most prominent schizophrenia risk genes [140]. Finally, alcohol affects the activity of microglia, and alcohol-induced microglial dysfunction has been proposed to contribute to the developmental effects of fetal alcohol exposure, in part through perturbation of synaptic pruning [141].

Altered Excitatory/Inhibitory Balance in Neurodevelopmental Disorders

A common theme emerging from studies of neurodevelopmental and neuropsychiatric diseases like autism and schizophrenia is that of an imbalance between excitation and inhibition [142, 143]. Epilepsy is more common among individuals with ASD than among the general population, consistent with hyperexcitability [144], and altered expression of GABA receptor subunits and enzymes involved in GABA synthesis has been reported in brains from autistic patients [145, 146]. Electrophysiological and biochemical characterization of animal models for monogenic CNS disorders including Fragile X and Rett syndrome have also provided evidence of altered E/I balance [147–149]. The causes of the alterations in E/I balance observed in autism and other disorders are not thoroughly understood but likely reflect impairments one or more of the synapse formation and remodeling processes described in this chapter.

Conclusion

Synaptogenesis involves precisely orchestrated interactions among a multitude of cells and proteins, and there is convincing evidence that developmental diseases of the CNS such as autism and schizophrenia involve a disruption of these interactions. Neurodevelopmental phenotypes may trace back to perturbations that affect the initial contact between presumptive synaptic partners, their mutual recognition, synapse assembly, maturation, or selective elimination, resulting in aberrant synaptic connectivity and E/I balance in the CNS.

It is important to note that, in addition to the many deficits in synaptogenesis described above, other neurobiological processes are almost certainly disrupted in developmental brain diseases, including progenitor cell proliferation, differentiation, migration, axon guidance, dendrite outgrowth, and mature synapse function. Thus, while these syndromes have common overlapping features at the structural and functional level, their underlying causes are undoubtedly heterogeneous, and a one-size-fits-all approach to treatment is unlikely to emerge. In the case of monogenic neurodevelopmental disorders [150–152], gene replacement and antisense inhibition experiments in mouse models have shown promise, and there is hope that similar therapies may be employed for human patients in the future. Other new and innovative approaches will likely emerge as we improve our understanding of the mechanisms underlying neurodevelopmental diseases.

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Multiple Choice Questions

Q1. A synapse may be formed between:
- A. An axon terminal and a dendritic spine
- B. An axon terminal and the dendritic shaft
- C. An axon terminal and the soma
- D. An axon and another axon

[Key: A, B, C, D]

Q2. Which of the following components detects calcium to trigger synaptic vesicle fusion during synaptic transmission?
- A. Synaptobrevin
- B. Synaptophysin
- C. Synaptotagmin
- D. Synapsin

[Key: C]

Q3. Which of the following are synaptic adhesion molecules?
- A. PSD95 and Shank3
- B. Neurexin and neuroligin
- C. SynCAM and NCAM
- D. Syntaxin and SNAP25

[Key: B, C]

Q4. Which of the following statements have been shown to be correct?
- A. Astrocytes can promote synapse formation by releasing thrombospondin 2 (TSP2)
- B. Some proteins can be synthesized locally in the dendritic spines
- C. The inhibitory GABAergic synapses are usually formatted on the dendritic shaft
- D. The fragile X mental retardation protein (FMRP) is a DNA-binding protein

[Key: A, B, C].

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Axonal Guidance

6

Mikaela Nevin, Janine Gallego, and David D. Eisenstat

Learning Objectives

1. Understand the importance of axonal guidance in the overall connectivity of the brain
2. Have a broad understanding of the complex nature of axonal guidance
3. Understand the concept of the four major guidance cues required for guidance
4. Gain knowledge underlying the signaling, receptors, and genes required for normal and abnormal axonal guidance

Highlights

- Axonal growth cones are guided by four major extracellular cues.
- The trajectory of axons is broken down into a series of small steps.
- Integrins play a key role as a cell surface receptor involved in axon adhesion and guidance.
- Robo receptors are transmembrane proteins that prevent axons from crossing back over the midline commissures.
- Ephrins play a similar role in the spinal cord constraining non-crossing axons to one side of the cord.

Introduction

An important step in building a functional nervous system is the formation of neural circuits. During development, neurons send out long processes called axons that will make connections with other neurons to form that neural circuits that allow for transmission of information between different neurons and regions within the nervous system.

For these neural circuits to be correctly assembled, it is important for axons to reach the correct target destinations. There are many billions of neurons in the adult brain, and each of these makes thousands of connections with other neurons. All of these connections must be precisely wired for the nervous system to function correctly. Despite the challenge, axons navigate toward their target cells in a highly directed manner and rarely make navigational errors.

The question of how this precision is accomplished was first raised by Ramón y Cajal, the father of modern neuroscience. In histological sections, Cajal observed that the axons elaborated by developing neurons were tipped by distinct amoeboid-like projections, which he termed growth cones.

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He proposed that the growth cone was somehow responsible for guiding the axon toward its target by responding to chemoattractive/chemotactic substances from cells lying along its path.

These observations turned out to be remarkably prescient. We now know that axonal growth cones are guided by four *major types of extracellular cues*: short-range or adhesive attraction, short-range repulsion, long-range attraction (positive chemotaxis; the original mechanism proposed by Cajal) and long-range repulsion (negative chemotaxis) [1] (Fig. 6.1). The axon shaft is laid down behind the growth cone as it navigates through the developing nervous system. The growth cone expresses guidance cue receptors that allow it to receive and integrate guidance information from the surrounding environment, and in response to the information it receives, it may retract, make a turn, or continue on the same path. In this way, the axon grows in a directed manner to its target. Intensive research over the past several decades has identified many molecular guidance cues and receptors involved in axon guidance (see Table 6.1), as well as the signaling events that occur in the growth cone downstream of these cues. Before we can discuss the molecular underpinnings of axonal guidance, it is first necessary to introduce the growth cone and how it moves.

Table 6.1 Summary of extracellular cues/ligands, receptors, and responses of the growth cone

Cue/Ligand	Receptor	Response
CAMs	Integrins	Dependent on integrin subunits expressed by growth cone
CAMs	Other CAMs	Context-dependent but often attractive (contact adhesion)
Netrins	DCC Neogenin	Attraction/outgrowth (commissural axons)
Netrins	UNC5	Repulsion (motor trochlear axons) Repulsion (dorsal root ganglion/somatosensory axons)
EphAs	Ephrin-As	Repulsion (retinal axons, ipsilateral spinal cord axons)
EphBs	Ephrin-Bs	Repulsion (ipsilateral spinal cord axons)
Draxin	DCC (competes with netrin for receptor binding)	Repulsion (commissural axons)
Slits	Robos	Repulsion (commissural axons)
Class 3 Semaphorins	Neuropilins and PlexinAs	Repulsion (commissural axons)
Class 6 Semaphorins	Plexins	Repulsion

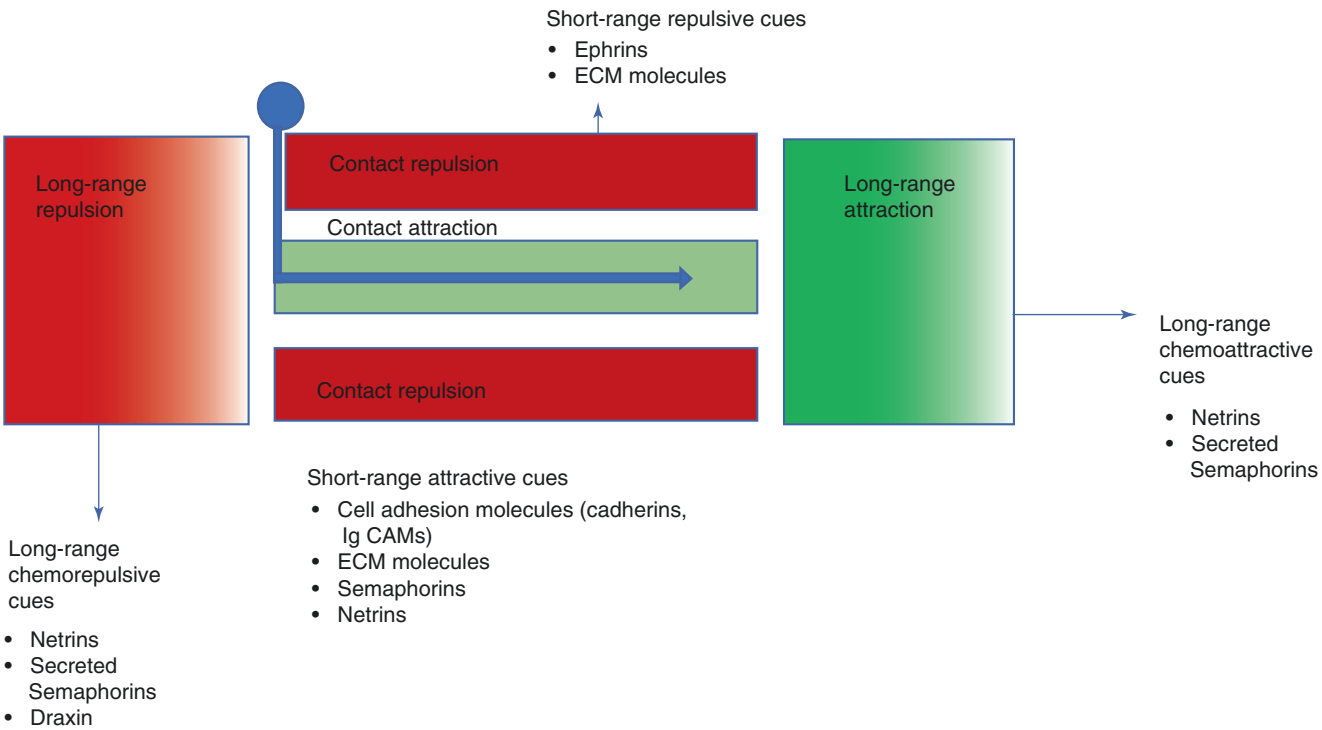


Fig. 6.1 Axons navigate with the help of four major types of guidance cues

The Growth Cone

The growth cone is a specialized fan-shaped structure at the distal tip of a growing axon (Fig. 6.2). The leading edge of the growth cone consists of two types of cytoskeletal projections: lamellipodia, which are flat, sheet-like structures that contain cross-linked actin filaments, and filopodia, which are finger-like projections composed of bundled F-actin. Just behind the leading edge (also known as the peripheral domain) is the microtubule-rich central domain of the growth cone.

The growth cone is driven forward by movement of its cytoskeleton. At the leading edge, continuous actin polymerization drives the protrusion of the filopodia and lamellipodia. There is a continuous retrograde flow of actin, driven by non-muscle myosin activity, from the leading edge to the more proximal central domain of the growth cone. Leading edge actin filaments also interact with non-muscle myosin to generate tractional forces, which help steer the growth cone and pull it forward. The balance between the rates of retrograde actin flow, actin polymerization at the leading edge, and actin depolymerization in the proximal region is what controls the movement of the growth cone. Broadly speaking, repulsive cues increase retrograde actin flow and decrease leading edge polymerization, which leads to growth cone retraction. Attractive cues decrease retrograde actin flow and increase leading edge polymerization, leading to advancement of the growth cone (Fig. 6.3). If a cue is presented asymmetrically to the growth cone, such as occurs when it is present in a gradient in the environment, then turning may result. The guidance cues that the growth cone encounters in its environment help steer it via downstream effects of signaling through their receptors on the activity of proteins that interact with the cytoskeleton and so alter cytoskeletal dynamics within the growth cone. Later in the chapter, we will learn more about some of the mechanisms that are involved in transducing the signal from an activated receptor on the growth cone membrane to the cytoskeleton.

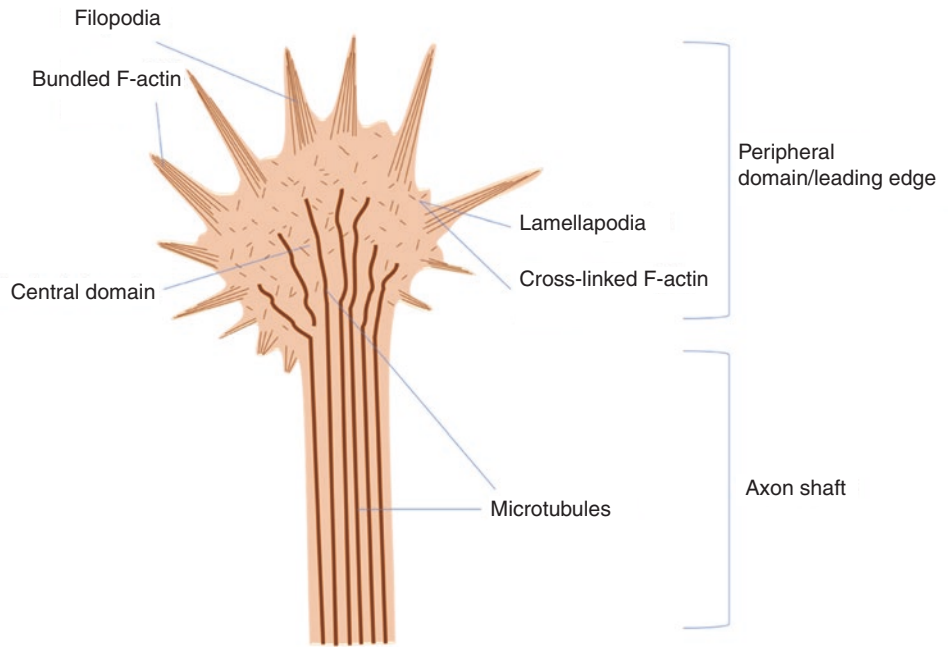


Fig. 6.2 The structure of the growth cone. The growth cone is a specialized extension of the growing axon that senses and responds to guidance information in the environment. Its leading edge consists of two types of cytoskeletal projections: lamellipodia, which are flat, sheetlike structures that contain cross-linked actin filaments; and filopodia, which are finger-like projections composed of bundled F-actin. Just behind the leading edge (or peripheral domain) is the microtubule-rich central domain of the growth cone

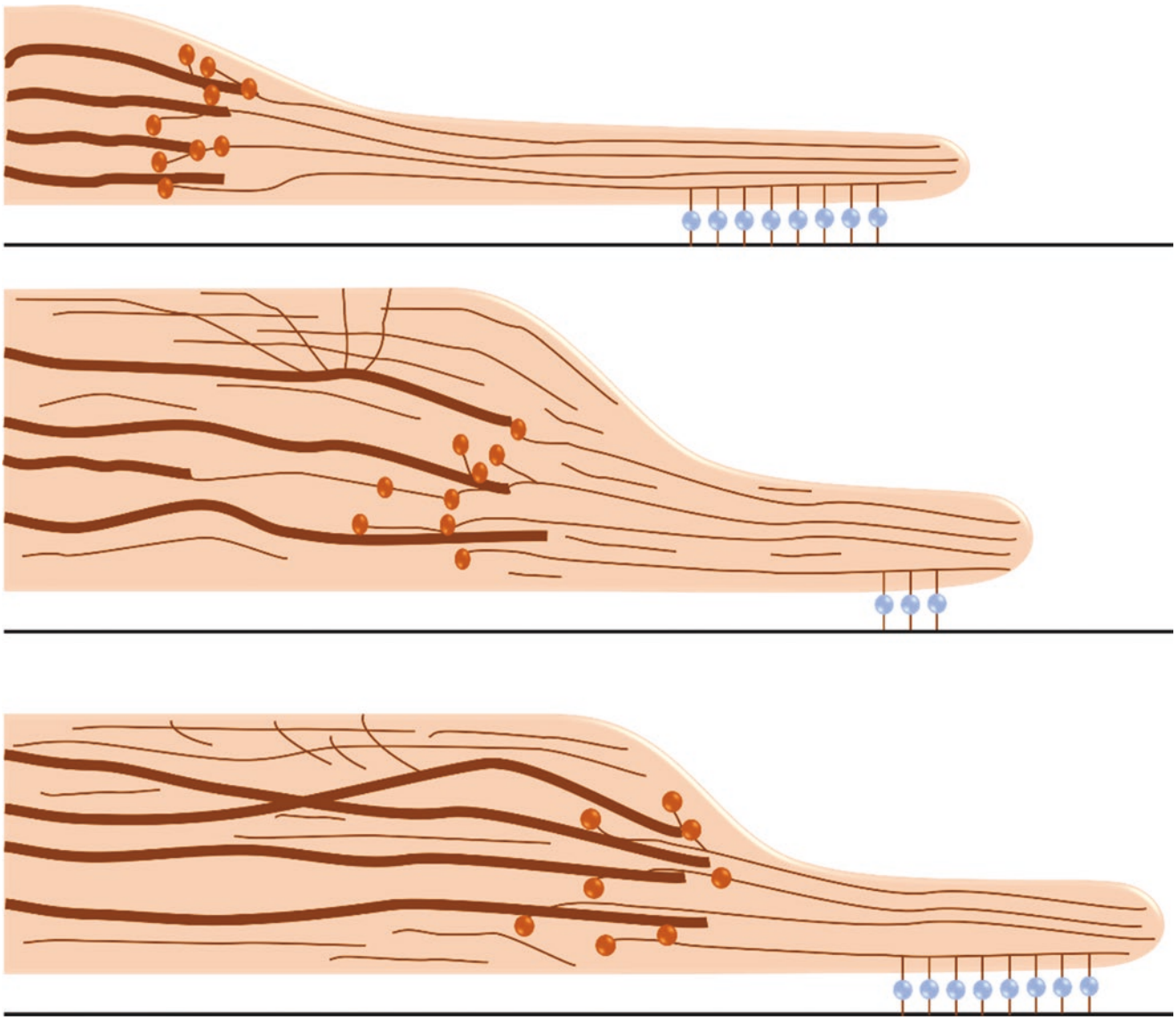


Fig. 6.3 Cytoskeletal dynamics in growth cone movement. Top: Continuous polymerization of actin at the leading edge drives the protrusion of the growth cone's lamellipodia and filopodia. At the same time, there is a continuous retrograde flow of actin from the leading edge toward the proximal domain of the growth cone. This is driven by the action of non-muscle myosin, which also interacts with the leading edge actin filaments to generate traction and pull the growth cone forward on the substrate. Middle: Repulsive cues increase retrograde actin flow and decrease actin polymerization at the leading edge, resulting in growth cone retraction. Bottom: Attractive cues decrease retrograde actin flow and increase actin polymerization at the leading edge, resulting in growth cone advancement

Role of the Substrate: Adhesive Guidance and Labeled Pathways

Just as a car needs a road to travel on, axons require a permissive substrate on which to adhere and extend. Many surfaces will not support axonal extension *in vitro* [2].

For extending axons, their "road" consists of molecules present in the extracellular matrix (ECM) and molecules present on the surfaces of surrounding cells and other axons (cell adhesion molecules, or CAMs). Interactions between these components is one mechanism by which short-range or contact attraction can be mediated. For an axon to adhere and extend on a given substrate, it needs to express the appropriate receptors on its surface. The primary class of cell surface receptor involved in mediating ECM-axon adhesion is the *integrin family*. Axons show preferences for extension on different ECM surfaces depending on which integrin receptor subtypes they express. However, because most ECM components are expressed in broad domains of the CNS, and some, like laminin, are expressed ubiquitously, it is thought that their main function in axon guidance is permissive rather than instructive. ECM-integrin

interactions may help constrain the axon to roughly the right area (i.e., keeping it on the road), but other guidance information is also necessary for the growth cone to get to the right destination. In some cases, CAMs and ECM components can play a more active role in axonal guidance beyond acting as a substrate for outgrowth. For example, the axons of commissural neurons in the spinal cord enter the floor plate (an important embryonic midline structure involved in guidance) partially due to interactions between two CAMs: the CAM TAG-1 expressed on the axons and NrCAM expressed by cells in the floor plate [3].

ECM components can also modulate the growth cone's response to a particular guidance cue, switching attraction to repulsion or vice versa. One example of this occurs during pathfinding of retinal ganglion cell (RGC) axons. RGC axons leave the eye because they are attracted to a molecule called netrin-1 at the optic nerve head. We will learn more about the netrins and the other classes of canonical guidance molecules later in the chapter. As the axons pass the optic nerve head, their response to netrin-1 switches so that they are now repelled by it. This change is induced by increased levels of the ECM molecule laminin-1 [4, 5].

Axons themselves can also help direct the extension of other axons. While the very first axons are growing in a largely axon-free environment that consists mainly of ECM and neuroepithelial cells, as development continues, the CNS becomes crisscrossed with many other axons. Later-extending axons can take advantage of this by growing on top of earlier-extending axons. This occurs via binding of CAMs, primarily cadherins and IgCAM members, on the surfaces of the axons. Axons that express the same CAMs will tend to bundle together, or fasciculate, as they grow. Large axonal tracts can be laid down in this way as later-extending *follower axons* expressing a particular CAM fasciculate with the earliest-extending *pioneer axons* expressing that same CAM. This is known as the *labeled pathways hypothesis*. At points where axons have to make decisions about where to go next—for example, whether to continue in the same fascicle or join a new one—it is often observed that they change which CAMs they express on their surface.

Intermediate Targets Simplify the Journey

The job of a pathfinding axon is also made easier by the use of *intermediate targets*. Many axons do not navigate directly to their final target location. Instead, their trajectory is broken down into a series of shorter steps. This simplifies the pathfinding task because it means that axons only have to navigate the short distances from one intermediate target to the next. This strategy ensures axons reach their correct final targets despite the complexity of the nervous system and the long distances they often have to travel. The question is now pushed back slightly: what guides axons from one intermediate target to the next? We have already seen how the growth substrate can affect where the axon grows. Besides this, intensive research efforts over the past several decades to understand the molecular basis of axon guidance have identified four major classes of guidance cue.

One structure that has already been mentioned and that we will encounter repeatedly in the following sections is the *floor plate*. The floor plate is a collection of specialized glial cells lying at the ventral midline of the vertebrate neural tube. For organisms that have bilaterally symmetric nervous systems, a critical guidance decision that axons must make is whether or not to cross the midline. Axons that cross the midline form commissural projections, which connect the left and right halves of the central nervous system and allow coordination and integration of activity from both sides. An easily recognizable example of a commissure in humans is the corpus callosum, which connects the left and right cerebral hemispheres. The floor plate secretes various guidance molecules that are involved in mediating the midline crossing decision in the developing spinal cord. There is a dorsal counterpart to the floor plate, the *roof plate*, which also provides guidance information to axons. These structures are also involved in patterning, specification, and differentiation of neuronal cell types via secretion of morphogens such as sonic hedgehog.

The floor plate is an important intermediate target for a very well-studied population of spinal cord axons, the axons of dorsal commissural interneurons. Several canonical guidance molecules were discovered through investigations into guidance events of commissural axons that occur at the floor plate. We will now learn about these molecules and discuss their involvement in some key guidance events at the floor plate.

Robo/Slit Signaling Prevents Commissural Axons from Recrossing the Midline

Robo receptors were first discovered in a screen for axonal guidance mutants in *Drosophila melanogaster* [6]. The name comes from the phenotype of the mutant flies. The organization of the *Drosophila* ventral nerve cord consists of two parallel axon tracts running longitudinally to the midline, crossed at regular intervals by commissural axons to create a repeating ladder-like pattern. Commissural axons normally cross the midline only once before turning longitudinally to continue along their trajectory. Ipsilaterally projecting axons never cross. In *Robo* mutant flies, all axons cross and recross the midline repeatedly, going around in circles. Because of this, the gene was named roundabout, or Robo.

The Robo receptors comprise a small family of single-pass transmembrane proteins. All Robo members have a

similar structure consisting of five immunoglobulin-like (Ig) domains, three fibronectin type III (FN3) repeats, and a transmembrane and cytoplasmic domain. There are four Robo family members in vertebrates, three of which are important in axon guidance: Robo1, Robo2, and Robo3/Rig-1.

The canonical ligands for Robos are the Slits. The Slits are a family of highly conserved secreted proteins. *Drosophila* has one Slit member, whereas vertebrates have three. Slits are secreted from specialized glial cells at the floor plate and bind Robo family members. At the floor plate, Slit-Robo binding repels axons and helps ensure that they do not recross. This was made clear by analysis of the phenotype of *Slit* mutant flies, in which commissural axons fail to leave the midline and collapse into a single longitudinal tract [7]. Commissural axon guidance defects are seen only in triple *Slit* knockout mice, indicating some degree of redundancy between the three Slit family members in vertebrates. [8].

Sensitivity of commissural growth cones to Slit-mediated repulsion must be temporally regulated in some way; otherwise, commissural axons would never be able to approach and enter the Slit-expressing midline. Robo expression on the growth cone membrane is carefully temporally and spatially regulated so that growth cones do not respond to the repulsive signal of midline Slits too early. Regulation occurs at the post-translational level. In Drosophila, this occurs through the action of a protein called Comm (short for commissureless). *Comm* was identified in the same mutant screen as *robo. Comm* mutants have the opposite phenotype to *Robo* mutants. In these flies, all commissural axons fail to cross the midline [6, 9].

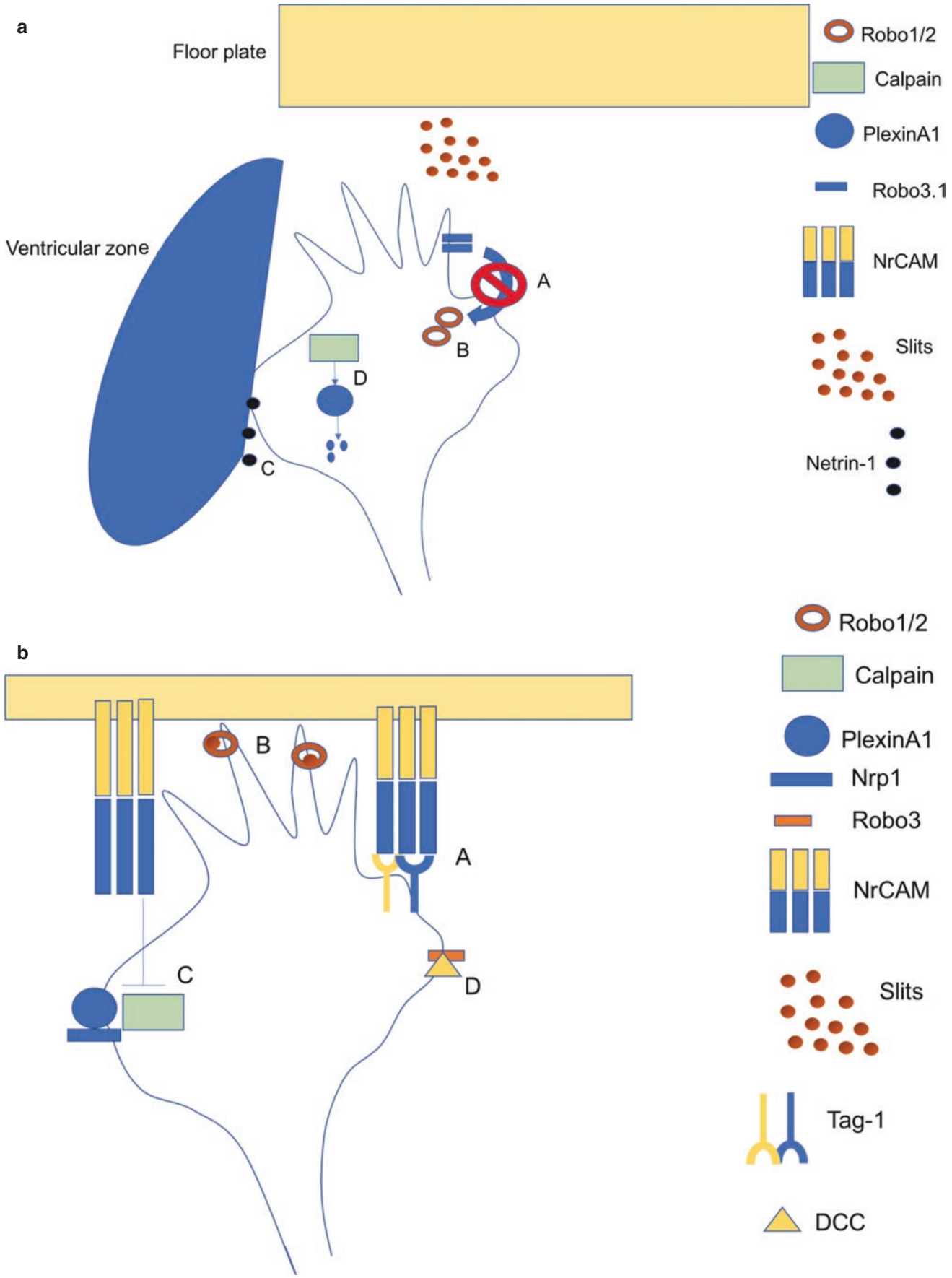
Comm prevents Robo expression on the growth cones of pre-crossing commissural axons by sorting it to the endosomal pathway for degradation [10]. Because they do not express Robo on their surface, the growth cones of pre-crossing commissural axons are insensitive to the repellent effect of midline Slits. This allows them to enter and cross the floor plate. After crossing, Comm expression is downregulated. The axons now become sensitive to Slit-mediated repulsion and are expelled from the midline. Ipsilaterally projecting axons express Robo highly at all times, and this prevents them from crossing.

However, vertebrates, do not have a *comm* homolog. So how is responsiveness to Slit signaling at the vertebrate midline regulated? One mechanism that has been proposed involves the divergent Robo member Robo3/Rig1 [11–13]. Robo3/Rig1 was first identified as a gene that was highly expressed in embryos mutant for Rb (retinoblastoma protein) [14]. It shares about 40% of its amino acid identity with other Robo family members. When its protein expression pattern was characterized, it was found to be highly expressed on both the pre- and post-crossing portions of commissural axons. In *Robo3* single knockout mice, all commissural axons fail to cross the midline [11, 12]. These findings suggested that Robo3 might not be involved in repelling commissural axons from the midline like the other two family members. Subsequent, more detailed expression studies revealed that there are two splice variants of Robo3: Robo3.1 and Robo3.2. Robo3.1 is highly expressed on the pre-crossing and crossing portions of commissural axons and is rapidly downregulated post-crossing. Robo3.2 is highly expressed on the post-crossing portion of the axon only [15].

Overexpression of Robo3.1 in chick commissural axons resulted in many axons recrossing the midline. Overexpression of Robo3.2 had the opposite effect: many axons failed to enter the midline. Reintroduction of Robo3.1 cDNA into *Robo3*-null mice (in which axons fail to cross) was able to rescue the axon guidance phenotype, whereas Robo3.2 introduction did not rescue this phenotype. A model that has been proposed to explain these results in vertebrates is that Robo3.1 helps prevent premature Slit-mediated repulsion of commissural axons at the midline, and Robo3.2 helps expel them from the midline once they have crossed. However, further mechanistic evidence for this model is required [16].

Examples of Robo/Slit interactions are illustrated in Fig. 6.4a, b.

Fig. 6.4 (**a**) Regulation of growth cone response to midline guidance cues: Pre-crossing. A. (Vertebrates) Robo3.1 prevents responsiveness to Slit-mediated repulsion via unknown mechanisms. B. (Invertebrates) Comm sorts Robo for degradation inside the growth cone, preventing response to midline Slits. C. Netrin1 deposited on the pial surface of the ventricular zone promotes growth cone adhesion and helps guide it toward the floor plate. D. Calpain degrades PlexinA1 inside the growth cone, preventing it from responding to floor plate-secreted class 3 Semaphorins. (**b**) Regulation of growth cone response to midline guidance cues: Crossing. A. Contact between NrCAM at the floor plate and Tag-1 on the growth cone axon promotes entrance of the growth cone to the floor plate. B. Slits bind Robo1/2 to help expel the growth cone from the midline. C. NrCAM inhibits calpain mediated degradation of Plexin A1. The growth cone can now respond to class 3 Semaphorins from the floor plate via Nrp2/Plexin A1 complexes expressed on its surface. D. Robo3 interacts with DCC to downregulate attractive response to Netrin-1 so the growth cone does not linger at the midline


a
Floor plate
Ventricular zone
A
B
C
D
Robo1/2
Calpain
PlexinA1
Robo3.1
NrCAM
Slits
Netrin-1
b
A
B
C
D
Robo1/2
Calpain
PlexinA1
Nrp1
Robo3
NrCAM
Slits
Tag-1
DCC

Semaphorin/Plexin/Neuropilin Signaling

The semaphorins (Semas) are a large conserved class of secreted and membrane-bound proteins. They are defined by the presence of a conserved cysteine-rich extracellular semaphorin domain [17]. Altogether, the semaphorin family consists of over 20 members subdivided into eight classes according to structure and phylogeny. The class 3–7 semaphorins are found in vertebrates. The first semaphorin was identified in a monoclonal antibody screen for molecules involved in selective fasciculation of axons in the developing grasshopper limb [18]. It was subsequently recognized that it was part of a larger conserved family of guidance molecules, which came to be called the semaphorins [19]. The first vertebrate semaphorin was purified from embryonic chick brain membranes based on its ability to collapse the growth cones of dorsal root ganglion cell axons in culture. Because of this property, it was initially named collapsin. Collapsin is now known as Sema3A [20].

The receptors for semaphorins are the plexins. Plexins are large transmembrane proteins that are evolutionarily related to the semaphorins, and also contain a sema domain. In vertebrates, there are four classes of plexins, Plexins A–D. in addition to plexins, the class 3 semaphorins also require an additional co-receptor, one of the two neuropilins, for signaling activity. The one exception is Sema3E, which binds Plexin D1 directly. The neuropilins are transmembrane proteins with short, non-catalytically active cytoplasmic domains. Together with the plexins, they form holoreceptor complexes for the class 3 semaphorins. Sema3 receptor complexes can also involve other components, which may help confer signaling specificity. For example, the Ig family member L1 associates with the Plexin A/Nrp1 complex and is required for axon response to Sema3A, but not for signaling via other class 3 semaphorins.

Class 3 Semaphorins Help to Expel Commissural Axons from the Floor Plate

Slits are not the only repellents acting at the midline. Here, Sema3B also contributes to repulsion of post-crossing axons. The cells of the floor plate express Sema3B, which signals through plexin A1 and neuropilin-2 expressed on the growth cones of post-crossing commissural axons. This helps to repel them from the midline. Why do not pre-crossing commissural axons respond to the Sema3B signal from the floor plate? Similar to Robo/Slit signaling at the midline, regulation of commissural axon responsiveness to Sema3-mediated repulsion is regulated at the post-translational level [21]. Pre-crossing axons are insensitive to Sema3-mediated repulsion because one of its receptor components, plexin A1, is degraded by the protease calpain in pre-crossing axons. When the axons contact the floor plate, calpain is inactivated by the floor plate CAM, NrCAM. The axons now express plexin A1 on their surfaces and can respond to the repulsive semaphorin signaling at the floor plate helping them to leave the midline [22].

An alternate model has been by another group that Nrp2 and plexin A1 are both actually expressed on commissural axons prior to crossing (previous results were an artifact produced by use of an insufficiently specific antibody). What prevents them from prematurely being repelled from the floor plate? Hernandez-Enriquez et al., [23] propose that there is an "Nrp2 sink." Since Nrp2 is also expressed on floor plate cells, this receptor can act as a molecular sink by binding Sema3B secreted from the floor plate. This binding prevents Sema3B from binding commissural axons before they have crossed. Later, Nrp2 is downregulated in floor plate cells through an uncharacterized mechanism. This frees Sema3B to bind commissural axons and repel them from the midline.

Examples of Semaphorin/Neuropilin/Plexin interactions are illustrated in Fig. 6.4a, b.

Ephrin/Eph Signaling

The Ephs comprise the largest class of receptor tyrosine kinases (RTKs) in the genome. They are grouped into A and B subclasses. Mammals have nine EphAs and five EphBs. The ligands for Ephs are the ephrins. The ephrins also are subdivided into EphrinAs and EphrinBs. EphrinAs are tethered to the membrane by a glycosylphosphatidylinositol (GPI) linkage, whereas EphrinBs have a transmembrane domain. EphrinBs additionally contain a non-catalytically active cytoplasmic tail. Eph/Ephrin signaling has several unique features compared to other RTKs. Both receptor and ligand are membrane-bound, meaning that Ephrin/Eph signaling requires cell–cell contact and likely only function as a short-range guidance cue. Ephrins and Ephs can also participate in reverse signaling where there is signaling into the ligand (ephrin) expressing cell upon receptor (Eph) activation.

EphrinBs and EphAs Constrain Non-Crossing Axons in the Spinal Cord

Ephrins are another class of cue implicated in the regulation of midline crossing. They have important functions in guiding several populations of axons in the spinal cord, mostly by constraining non-crossing axons to one side of the spinal cord. Ephrin B3 expressed on glial cells at the dorsal and ventral midlines forms a repulsive barrier for EphA4-expressing ipsilateral axons and ensures that they do not cross [16]. In *EphA4* and *Ephrin B3* null mice, these axons

inappropriately enter the midline. Axons of the corticospinal tract (CST) also express EphA4. Interaction with Ephrin B3 at the dorsal midline prevents them from recrossing after they decussate at the medulla [16, 24, 25]. Mice in which *Eph4A* or *EphrinB3* functions are genetically disrupted and show the same neurological phenotype, consisting of an abnormal hopping gait. This phenotype is also seen in mice lacking the GTPase-activating protein N-chimerin, which is a known downstream effector of EphA4 signaling. The cause of this phenotype is ventral midline crossing of EphA4-expressing excitatory axons. Normally these axons do not cross the midline and only innervate ipsilateral motor neurons. Innervation of bilateral and ipsilateral motor neurons by these axons presumably disrupts unilateral motor control and leads to the abnormal gait. Lastly, ephrinB3-EphA4 interaction also prevents ascending ipsilateral spinal cord axons from entering the dorsal midline.

Mapping of Retinal Axon Projections by EphrinA Signaling

Ephrin-A/EphA signaling are also important in the guidance and topographic mapping of retinal axons and their role in axon guidance was first described in this system [26, 27]. The target of retinal axons is an area of the midbrain called the optic tectum. Retinal axons project to this area based on their area of origin in the retina. Temporal axons project to the anterior optic tectum, and nasal axons project to the posterior part. *In vitro,* temporal retinal axons prefer to grow on anterior tectum membranes and will avoid posterior tectum membranes. Biochemical studies indicated that the repulsive factor was likely a membrane-bound protein. It was also found that the repellent was distributed in a graded manner in the tectum, being highest posteriorly (caudally) and lowest anteriorly (rostrally) [28, 29]. EphrinA5 and EphrinA2 were subsequently identified as the repellent factors.

EphrinA5 and EphrinA2 are expressed in a posterior–anterior high-low gradient in the optic tectum. Along the nasal-temporal axis of the retina, EphA members are expressed on RGC axons in a complementary gradient [30]. EphrinA-mediated repulsion and competition between RGC axons restrict those axons expressing low levels of EphA members to regions with high EphrinA levels, and vice versa [31, 32]. Thus, temporal axons (EphA-high) avoid the posterior part of the tectum where EphrinA levels are the highest.

Ephrin-A5 null mice show defects in topographic mapping of temporal and nasal retinal axons. Temporal axons of these mice project more posteriorly within the superior colliculus (SC) than they should. Some retinal axons also overshoot their normal target and even extend all the way to the inferior colliculus (IC) (although anterograde labelling of axons from the temporal retina at postnatal time points revealed that these abnormal projections are not maintained). The *EphrinA2/A5* double knockout mouse shows more extensive mapping defects, with the normal anterior–posterior ordering of temporal and nasal retinal axons being severely disrupted. It is not completely lost, indicating that other guidance cues besides EphrinAs also are involved in guidance and mapping of nasal and temporal retinal axons [32, 33].

Ephrin-A5 in the IC also helps restrict retinal axons to the SC [29]. EphrinA5 induces collapse of and repels retinal axon growth cones *in vitro* [34]. Ephrin forward signaling initiates activation of intrinsic tyrosine kinase activity. Subsequently, there is tyrosine phosphorylation of various effector proteins in the growth cone. Effector proteins regulate the activity of various Rho GTPases, e.g., Rac1, Cdc42, RhoA resulting in a downstream effect on cytoskeletal dynamics.

Examples of Ephrin/Eph interactions are illustrated in Fig. 6.4a, b.

Netrins

Netrins Attract Commissural Axons to the Midline

The netrins are a family of extracellular proteins belonging to the laminin superfamily. They are best studied for their role in guidance of spinal interneuron commissural axons. The first vertebrate netrins, Netrin-1 and Netrin-2, were identified and purified from embryonic chick brain extracts based on their ability to promote outgrowth of spinal commissural interneuron axons *in vitro* [35, 36]. Subsequently, it was shown that they could also reorient the growth cones of these axons *in vitro* in addition to promoting their outgrowth [35]. Expression studies suggested that Netrin-1 was present in a gradient at the floor plate, with the highest concentrations being closest to the floor plate. The trajectories of commissural axons in *Netrin1* mutant mice were profoundly abnormal and disorganized, and many failed to reach the floor plate [37]. Although the original *Netrin*1 mutant was a severe hypomorphic allele rather than a true knockout, subsequent studies in the full knockout mouse have shown that in the absence of Netrin, axons fail to cross the midline, confirming the importance of this molecule for commissural axon guidance. Together, these findings led to the classical model of *Netrin-1* function in which a gradient of Netrin-1 from the floor plate acts at a long range to attract commissural axons to the midline.

However, this model did not fully account for certain observations about the Netrins. For example, earlier studies in Drosophila showed that that midline commissures formed normally in embryos that were genetically engineered to

express only a membrane-tethered form of netrin. This suggested that a diffusible gradient of netrin is not actually required to guide commissural axons [16]. More recent experiments have taken advantage of the *Cre-lox* system to generate conditional knockout mice in which *Netrin1* expression was ablated only in the floor plate or progenitor cells of the ventricular zone (VZ) of the spinal cord [38, 39]. These elegant experiments revealed that Netrin1 expression in the floor plate may be dispensable for commissural axon guidance. Commissural axons still approached and crossed the midline in mice lacking *Netrin1* expression only in the floor plate. In contrast, *Netrin1* deletion only in the VZ resulted in commissural axon guidance defects similar to those see in *Netrin1* knockout mice. These findings have led to a revised model of Netrin1 signaling at the floor plate: ventricular zone progenitor cells deposit Netrin1 at the pial surface of the spinal cord and hindbrain, where it acts at a short range to promote adhesion of commissural axon growth cones and guide them across the midline.

Netrins can also function as repellents for other classes of axons. Netrin-1 been shown to repel trochlear motor axons *in vitro* [40], and furthermore Netrin-1 in the ventral spinal cord repels sensory axons, promoting their dorsal extension [16].

Whether Netrins elicit a repulsive or attractive response depends on which netrin receptors are expressed on the growth cone. The attractive effect of Netrin is mediated through interaction with the DCC (Deleted in Colorectal Cancer) receptor, whereas Netrin-mediated repulsion requires expression of the UNC-5 receptor, the vertebrate homolog of the *C. elegans unc-5* (uncoordinated) gene.

A more recently discovered guidance cue that functions in the Netrin signaling pathway is *Draxin* (Dorsal Inhibitory Axon Guidance Protein). Draxin is a secreted molecule without homology to other known guidance cues. Current evidence suggests that it acts as a Netrin-1 antagonist [41]. Draxin has a repulsive/growth-cone collapsing effect on some populations of spinal commissural axons and on axons from cortical explants *in vitro* [42]. *Draxin* knockout mice show a complete absence of forebrain commissures and defasciculation of spinal commissural axons [42]. Draxin from the roof plate also helps repel commissural axons so that they move toward the ventral midline [21].

The netrins are often brought up as a prototypical example of a bifunctional axonal guidance cue. In fact, it is important to realize that guidance cue response is a dynamic and context-dependent process. All cues can mediate attraction or repulsion depending on the external environment, the internal environment of the growth cone, and which receptors the growth cone expresses on its surface. In the next section, we will learn about some of the mechanisms used by growth cones to modulate their responses to a given cue.

Examples of Netrin/DCC/UNC5 interactions are illustrated in Fig. 6.4a, b.

Response-Switching Regulatory Mechanisms

The trajectory of most axons is broken down into a series of smaller steps in which axons are guided from one intermediate target to the next, rather than travelling directly from their originating neuron to their final target. This strategy helps ensure accurate pathfinding across long distances but presents a new problem: axons must switch their response to a given guidance cue at each choice point or intermediate target, or they will not be able to move away from it.

Response-switching occurs by changes in guidance cue receptor expression on the growth cone.

Changes in receptor expression are also required to prepare the growth cone for the next stage of their journey where they may encounter different guidance cues than they did before. Guidance cue receptor expression can be regulated at the transcriptional, translational, or post-translational levels. We have already seen some examples of post-translational regulation in the section dealing with Robo signaling [21]:

- *Transcriptional*: Initiation or cessation of guidance cue expression (at the transcriptional level) frequently coincides with a growth cone's arrival at a choice point/intermediate target.
- *Translational*: One way of rapidly regulating receptor expression is via *local translation* of guidance receptor mRNA within the growth cone itself. This allows faster response-switching than translation occurring in the neuronal cell body, since locally translated guidance receptors do not have to be translocated down the axon shaft before being inserted on the growth cone membrane. Local translation has been shown to be important for signaling through multiple guidance pathways. For example, Netrin-1 induces local translation of one of its own receptors, DSCAM, in growth cones [43].
- *Post-translational*: Receptor expression can also be regulated post-translationally. Membrane insertion of guidance receptors can be modified at the level of multiple processes: receptor trafficking or sorting, removal of guidance receptors from the growth cone surface via proteolytic cleavage, or via modulatory interactions with other guidance receptors in cis (in the plane of the same membrane) or in trans (on other cells). The termination of Eph/Ephrin signaling provides examples of some of these processes. Eph/Ephrin signaling requires cell–cell contact, since both components are membrane-bound. This raises the question of how Eph/ephrin-mediated repulsion can occur, because initiation of the signal requires a high-affinity adhesive interaction between two cells. Two mechanisms have been shown to be involved in this process: ectodomain shedding and endocytosis [44, 45]. Ectodomain shedding in Ephrin signaling involves the protease KUZ (Kuzbanian; the *Drosophila* homolog of

the metallopeptidase ADAM10) which forms a complex with EphrinA2. When an Eph interacts with EphrinA2, ADAM10 cleaves the EphrinA2/Eph complex, terminating the repulsive signal.

Regulatory Mechanisms Not Involving Changes in Receptor Expression in the Growth Cone

- *Cis* interactions with other receptors expressed on the growth cone membrane can modify the way the growth cone responds to a cue. For example, during midline crossing, Robo3 interacts *in cis* (on the same membrane) with the Netrin receptor DCC to attenuate the response to Netrin-1 [13]. This mechanism helps ensure downregulation of Netrin-mediated attraction so the growth cone can leave the midline.
- *Receptor crosstalk* with other guidance signaling pathways can modify the response as well. For example, the morphogen sonic hedgehog (Shh) can induce sensitivity to class 3 semaphorins [21, 46].
- *Intracellular calcium and cyclic nucleotide levels*. Two particularly important components of the intracellular environment that modulate guidance cue response are calcium and cyclic nucleotide levels, respectively. An attractive response can often be converted to a repulsive one when cAMP or cGMP levels are lowered in the growth cone [5, 47]. For example, axons of retinal ganglion cells are initially guided out of the eye by the attractive effects of Netrin-1 at the optic nerve head [48]. However, as these RGC axons continue along the optic nerve, this attractive response changes to repulsion due to decreased cAMP levels within the growth cone. The change in cAMP levels is partially intrinsic [48] and partially a result of increased levels of laminin-1, as mentioned previously [4, 5].

Intracellular Signaling in the Growth Cone

When a guidance molecule binds its receptor on the surface of the growth cone, a cascade of events takes place within the growth cone to elicit a response. Receptor engagement triggers a series of downstream events in the growth cone that culminate in modulation of cytoskeletal dynamics. This results in growth cone retraction, advancement, or turning.

Activated receptors expressed on the surface of the growth cone either directly activate other effectors or recruit other proteins that do so. These downstream effectors modulate guanine nucleotide exchange factors (GEF) activity, with resulting changes in Rho GTPase activity that moderate the activity of various proteins that control cytoskeletal processes, including cofilin and polymerization of actin filaments. Cofilin activity leads to alterations in cytoskeletal dynamics. Reduced actin filament polymerization results in alterations in growth cone movement, morphology, and attachment to the substrate.

The first step in this process is activation of the receptor by guidance cue binding. The activated receptor subsequently modulates the activity of further downstream effectors, such as kinases, either directly or indirectly. In some cases, such as the Ephs, this process is direct because the guidance receptor itself has a cytoplasmic domain with intrinsic kinase or phosphatase activity. In other cases, the activated receptor recruits additional cytoplasmic effectors to transduce the signal. For example, when Robo receptors are activated by Slit binding, Slit-Robo *GTPase-activating proteins* (srGAPs) are recruited to the receptor complex. Activity of srGAPs results in inactivation of the *Rho GTPase Cdc42*. In general, the Rho family GTPases are the key participants coupling guidance receptor activation to cytoskeletal rearrangement (Table 6.2). Guidance receptor signaling moderates Rho GTPases by modulating the activity of GEFs and GTPase activating proteins (GAPs). GEFs activate Rho

Table 6.2 Downstream signaling events in the growth cone

Receptor	Ligand	Effects on Rho and Ras GTPase activity	Other intracellular effectors	Cytoskeletal effects	Response	References
DCC	Netrin-1	Rac1 and Cdc42 activation	Src, Fyn		Growth cone attraction	[49], [50]
UNC5	Netrin-1	RhoA activation	cGMP, Src, Shp2		Growth cone repulsion	[49], [51]
Robo1	Slit	Cdc42 inactivation	Ena Profilin			[49]
Ephs/EphA members	Ephrins/ EphrinAs	RhoA activation; Rac2 and Cdc42 inactivation Ras inactivation	Ephexin PAK inactivation ABL dephosphorylation MAPK inactivation		Growth cone retraction	
Plexin A/ neuropilins	Class 3 semaphorins	Rac1 activation	LIM kinase activation Cofilin phosphorylation	↓ Actin filament turnover	Growth cone collapse	[52]

GTPase signaling by stimulating exchange of GTP for GDP. GAPs turn off Rho GTPase signaling activity by activating their intrinsic GTPase activity [44]. Rho GTPases modulate cytoskeletal dynamics by regulating downstream effectors that control processes such as actin polymerization, nucleation, depolymerization, and non-muscle myosin activity. Consequently, there is alteration of the growth cone's morphology, movement, and attachment to the substrate.

Axon Guidance in Human Disease

Only a few, rare human diseases resulting from defects in axon guidance have been described so far [53]. Table 6.3 lists some examples of human disorders that result from mutations in guidance molecule genes. It is worthwhile to note that most guidance cues are also involved in other developmental processes such as neuronal migration and axonal sprouting. Consequently, it is often unclear whether the phenotypes associated with mutations in guidance molecules are due to disruption in axonal pathfinding per se rather than other functions of these molecules.

For example, mutations in the semaphorin family member SEMA3A have been identified in some patients with *Kallmann Syndrome* (KS). KS is a form of hypogonadotropic hypogonadism that results from a failure of gonadotropin-releasing hormone (GnRH) producing neurons to migrate from their birthplace in the olfactory bulb to the hypothalamus during development. These neurons travel along the olfactory axons to reach the hypothalamus, so, in principle, genetic defects that disrupt either olfactory axon pathfinding or directly disrupt GnRH neuron migration could result in KS. Nevertheless, there is clear evidence of axon pathfinding defects in some of these diseases. For example, magnetic resonance imaging (MRI) of patients with *Horizontal Gaze Palsy with Progressive Scoliosis* (HGPPS) demonstrate that corticospinal and somatosensory tract axons are abnormally ipsilateral, indicating that these axons failed to cross the midline during development.

Single nucleotide polymorphisms (SNPs) and copy number variants (CNV) in several of the genes encoding guidance molecules have also been described in patients with autism, epilepsy, and several neurodegenerative diseases, including amyotrophic lateral sclerosis [57].

Table 6.3 Examples of diseases associated with defects in axonal guidance molecules

Disease	Gene(s) implicated	Axonal populations implicated	References
Horizontal gaze palsy with progressive scoliosis (HGPPS)	ROBO3	Axons of the corticospinal and somatosensory tracts	[54]
Kallman syndrome	SEMA3A	Olfactory axons	
L1 syndrome (encompasses X-linked hydrocephalus with stenosis of the aqueduct of Sylvius (HSAS); MASA syndrome; Spastic paraplegia type 1; and X-linked complicated corpus callosum agenesis)	L1CAM		
ACOG syndrome (agenesis of corpus callosum, axon pathfinding, cardiac, ocular, and genital defects)	CDH2 (encodes N-cadherin)	Corpus callosum, others	[55]
Congenital mirror movement disease	DCC		[56, 57]

Axonal Regeneration

Guidance molecules serve many other functions beyond growth cone guidance, and many of them continue to be expressed in the CNS after development is complete. Evidence is accumulating that axon guidance molecules may contribute to the failure of axonal regrowth that is seen in the injured adult CNS. For example, the Semaphorins, many of which function as repulsive cues for various populations of axons during development, continue to be expressed in the adult brain and spinal cord. Their presence in the CNS may be inhibitory for axonal outgrowth following injury. Guidance molecules are implicated in many other biological processes besides axon guidance, such as immune function and vascular development. These processes can also impact regeneration [58].

Summary

- Axonal trajectories are determined by four major types of extracellular guidance cues: short-range attractive, long-range attractive, short-range repulsive, and long-range repulsive (Fig. 6.1).
- Later-born axons use early-extending pioneer axons as a scaffold to grow to their correct targets.
- Axons use intermediate targets to simplify pathfinding.
- These interactions are fine-tuned and modified by other components of the environment, such as extracellular matrix components, and by complex interactions with other signaling pathways and receptors on the growth cone membrane.

- Guidance receptor expression is precisely controlled by a variety of mechanisms, allowing the right axons to respond to the right cues at the appropriate time and place.
- Guidance cues also participate in other developmental processes such as neuronal migration.
- Disruption of axonal pathfinding can contribute to human disease.

Multiple Choice Questions

Q1. **Which of the following cues are necessary for guidance of the axonal growth cone to its target?**
   A. Short-range attractive cues
   B. Long-range attractive cues
   C. Short-range repulsive cues
   D. Long-range repulsive cues
   E. A, B, and C
   F. All of the above

Q2. **Which of the following ligand/receptor pairs used during growth cone guidance is INCORRECT?**
   A. Slit/Robo
   B. Sema/Plexin
   C. Netrin/DCC
   D. Netrin/Plexin
   E. Eph/Ephrin

Q3. **All of the following downstream molecules have been implicated in Netrin-mediated signaling EXCEPT:**
   A. FAK
   B. SRC
   C. mTOR
   D. Rac1
   E. RhoA

Q4. **Which of the following mechanisms have been implicated in response-switching during growth cone guidance?**
   A. Gene transcription
   B. Local translation
   C. Ectodomain shedding
   D. Trans-interactions
   E. A, B and C
   F. All of the above

Q5. **Which human gene is NOT associated with the corresponding neurological disease?**
   A. Slit1/Horizontal gaze palsy with progressive scoliosis
   B. SEMA3A/Kallmann syndrome
   C. L1CAM/X-linked hydrocephalus
   D. CDH2/ACOG syndrome
   E. DCC/congenital mirror movement disease

Answers: 1F, 2D, 3C, 4F, 5A.

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Myelination

7

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Learning Objectives

1. Understand the underlying mechanism of electrochemical signaling in the brain
2. Know the structure and function of myelin
3. Have a basic understanding of the development and maturation of myelin
4. Obtain a basic understanding of pathologic models for abnormal myelinogenesis and/or destruction

Highlights

- Oligodendrocytes are the cells in the central nervous system responsible for the production and formation of myelin.
- Myelinogenesis occurs in waves that follow neurogenesis.
- A number of genes are responsible for oligodendroglial development and myelination. (*Olig1, Olig2, PDGFRa, Sox10, Nkx2.2, MBP, Myt1, Cnp,* and *Plp1).*
- Several genetic rodent models are now utilized for the better understanding of disorders of myelination.

Introduction

For the nervous system to function properly, rapid transmission of electrical signals between its different regions is necessary. To help achieve rapid electrical conduction, many central nervous system (CNS) axons with a diameter 0.2 mm or greater are ensheathed in lipid-predominant myelin, which acts as an electrical insulator to enable a rapid form of conduction termed saltatory conduction. Myelin in the CNS is produced by specialized cells called oligodendrocytes (OLs) that ensheath the axon and elaborate myelin as complex extensions of their plasma membranes. In the peripheral nervous system (PNS), myelin is produced by Schwann cells but similarly form tightly coiled myelin sheaths. A variety of neurological conditions can occur when myelination fails to form, contains abnormal components, or are lost from trauma, inherited or acquired disease. This chapter will outline CNS myelination processes, OL development, demyelinating diseases, and remyelination processes.

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Myelin: Structure and Function

Function

Myelination is the process of production of myelin sheaths around neuronal axons. The formation of the myelin sheath in the CNS is a crucial part of neurodevelopment and allows for normal function of the neuronal circuits [1]. Myelin is responsible for increased and more efficient electrical conduction through an axon by increasing resistance and decreasing capacitance [1, 2]. Myelin is responsible for the difference between dull and sharp pain sensations which are conducted by unmyelinated axons and myelinated axons, respectively (Table 7.1). An increase in myelin corresponds to increased conduction speed since the sheath acts as an electrical insulator that prevents ions responsible for electrical conduction (ex. Na^+ and K^+) from leaking out of the axon (Fig. 7.1). Action potential generation, which can be time-consuming, only has to take place at specific, unmyelinated points along the axon between the myelin sheaths called the Nodes of Ranvier. Proper positioning of these channels is essential for rapid conduction and control of aberrant neuronal firing throughout the CNS and PNS [3]. These nodes contain Na/K ATPase, sodium and potassium ion channels which allow action potential replenishment to occur, while the myelinated segments allow current to flow passively until it reaches the next node [3, 4]. The process is repeated along the axonal length and allows the current to "jump" from node to node rather than continuously regenerate along the entire axonal length.

Structure

In the CNS, myelin is considered an extension of the OL plasma membrane (Fig. 7.1) [2, 5]. The OLs extend their processes ending in concentric sheets of myelin and spirally wrap the sheet around neuronal axons leading to the multilamellar structures that are responsible for determining the speed of neuronal signals [2, 5, 6]. The cytosol is largely absent in the compacted areas of the myelin sheath but is present in the noncompacted regions to facilitate protein movement. These areas are the inner loop, outer loop, and paranodal loop, respectively (Fig. 7.2). Unlike the PNS where each myelin sheath is constructed by an individual Schwann cell that myelinates a single axon, OLs can produce up to 80 myelin sheaths on multiple axons [4, 7, 8]. Due to the large surface area of myelin—approximately $1–20 \times 10^5$ μm^2 in an adult rat—OLs must produce roughly 5000 μm^2 of myelin a day during active myelination.

The insulating function of myelin is largely reflected in its highly lipid structure which makes up approximately 70% of its weight [2, 9]. The 70% lipid content is broken-down into 32% glycolipid (majority galactocerebroside, GalC, and sulphatide), 26% cholesterol, and 42% phospholipid. Mice that are unable to produce myelin galactolipids GalC and sulfatide have been shown to have issues in axo-glial interactions—necessary for proper myelin formation and axolemmal organization—resulting in morphological abnormalities in the distribution of nodal and paranodal regions [3, 10].

Along with lipids, myelin consists of several proteins. The most prominent protein is the proteolipid protein PLP1 and its spliced isoform DM20 which makes up 50% of the protein mass. Thirty to forty percent of the CNS myelin protein mass is made up of myelin basic protein (MBP) which is a cytoplasmic, extrinsic membrane protein. Four percent of the myelin proteins is formed by 2′,3′-cyclic nucleotide 3′-phosphohydrolase (CNP) which is located on the cytoplasmic face of the membrane of OLs. Other proteins are myelin-associated glycoprotein (MAG) and myelin oligodendrocyte glycoprotein (MOG) that are suggested to have roles in initial axon-myelin interactions and myelin maintenance, respectively. Should any of the genes encoding these myelin proteins be disturbed, axonal degeneration occurs even when the myelin sheaths are structurally normal or only show minor changes [11]. These proteins are known to have non-structural roles and will be discussed later in this chapter.

Table 7.1 Properties of fiber types

Fiber type	Class	Function	Fiber diameter (mm)	Conduction velocity (m/s)	Myelination
Muscle					
Motor	Aα	Muscle contraction	12–20	70–120	Yes
Preganglionic efferent	B	Autonomic preganglionic	<3	3–15	Yes
Muscle spindle	Aγ	Muscle spindle afferent	3–6	15–30	Yes
Touch					
Cutaneous afferent	Aβ	Touch, cutaneous pressure, vibration	5–12	30–70	Yes
Pain					
"Fast" pain	Aδ	Pain, temperature, Cold sensation, localized touch, afferent	2–5	12–30	Yes
"Slow" pain	C	Pain, hot sensation generalized touch, afferent	0.4–1.2	0.5–2.0	No

Summarization of fiber type, class, function, fiber diameter, conduction velocity, and myelination. Positive relationship between the myelination and increased fiber conduction velocity [85, 86]

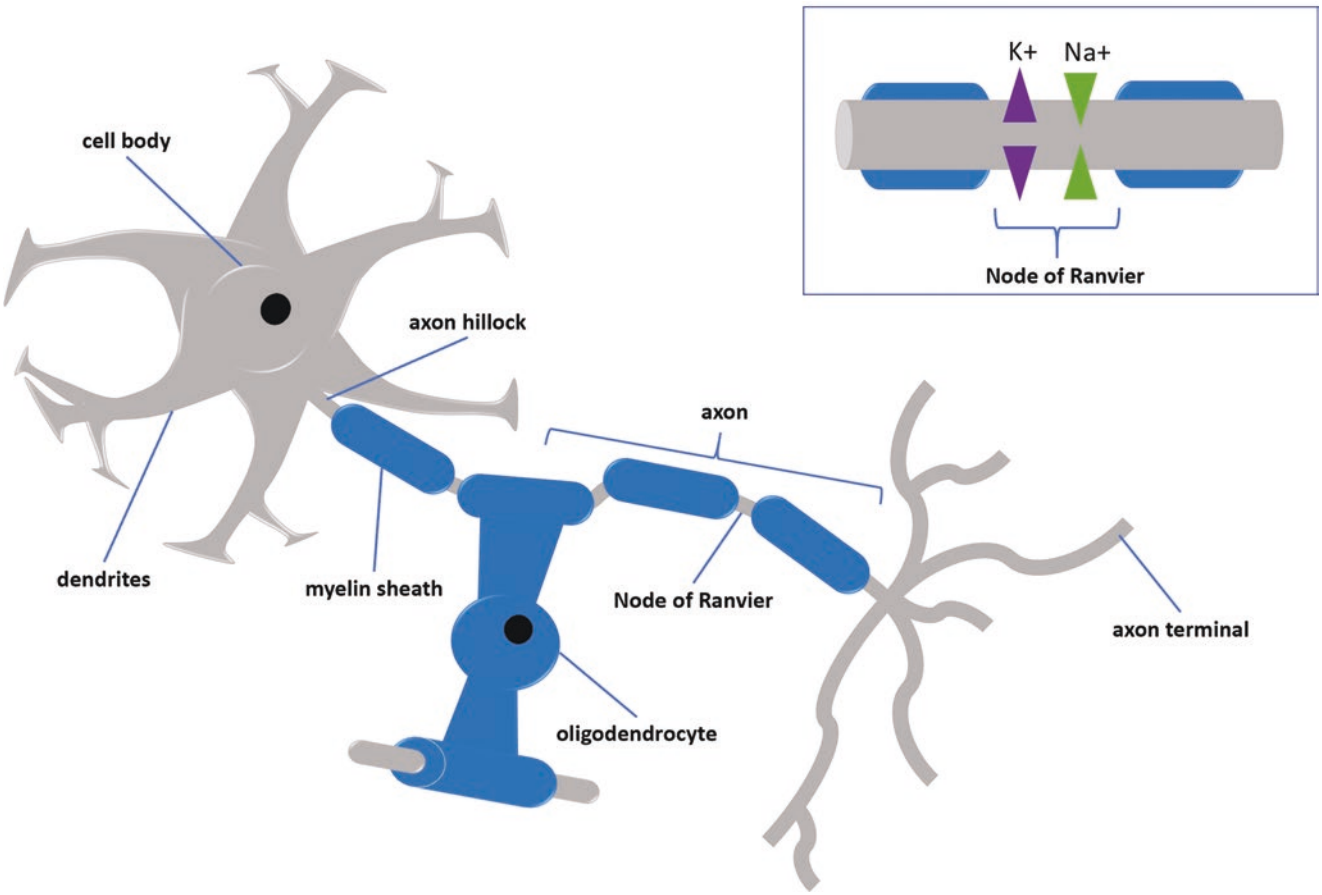

Fig. 7.1 Neuron structure. Oligodendrocyte projections wrap around neuronal axons with the Nodes of Ranvier present between each myelin sheath. Nodes of Ranvier contain Na^+, K^+ and other channels that replenish the axon potential. Na^+ concentration is higher outside the axon than inside while K^+ concentration is higher inside the axons than the outside during resting membrane potential (−70 mV). Na^+ ions enter and K^+ ions exit the axon following the electrochemical gradient

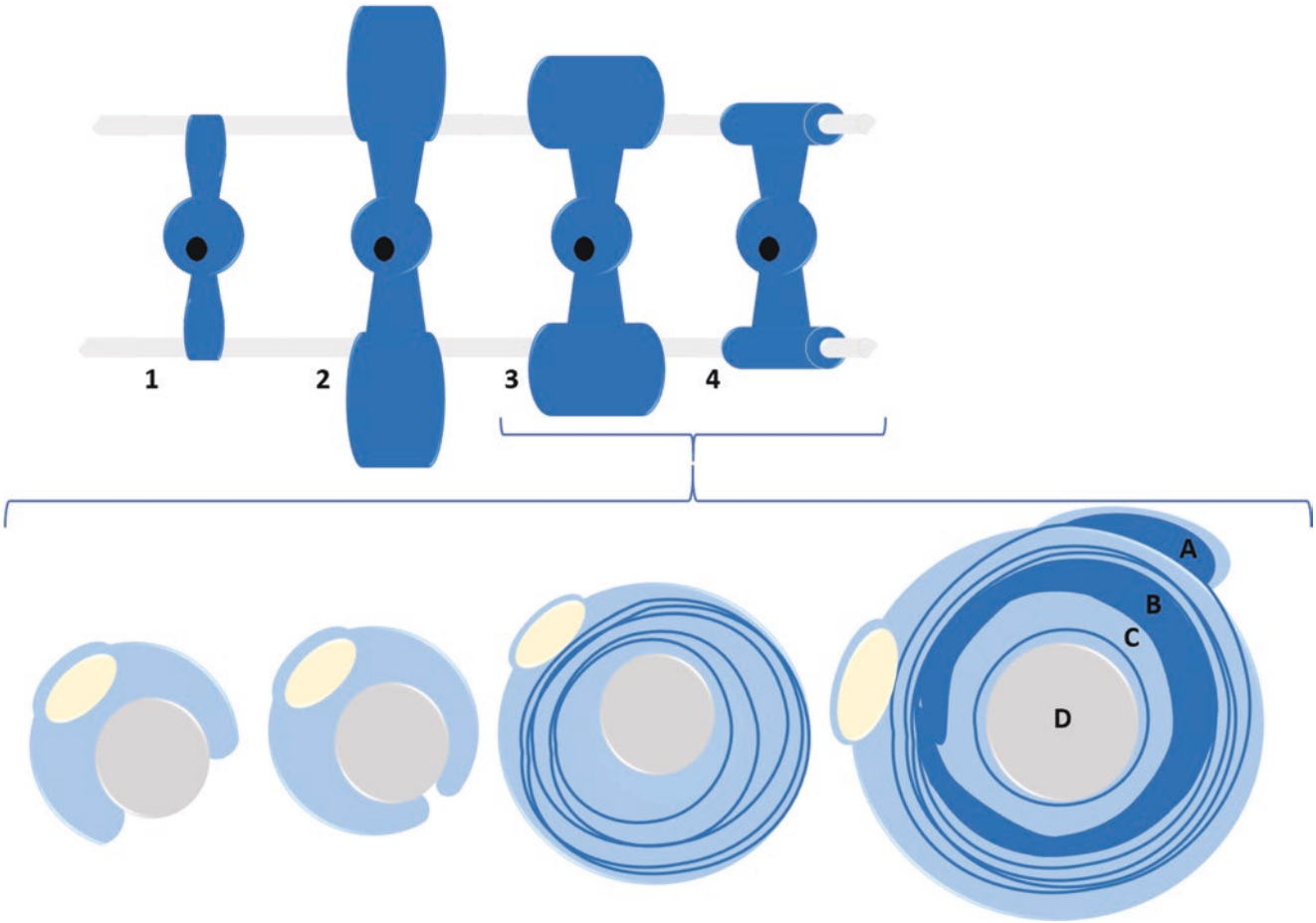

Fig. 7.2 Myelin structure and formation. (1) Recognition of axons; (2) Extension of projections and the myelin sheet; (3) Spiral wrapping of the myelin sheet around the axons with formation of the myelin sheath; (4) Compaction of the myelin sheath. A: outer loop, B: inner loop, C: periaxonal space, D: axon

Myelinogenesis

Myelinogenesis is the developmental process in which myelin protein and enzyme genes are simultaneously expressed leading to the production of myelin proteins and lipids [12]. In rodents and humans, this process occurs postnatally. In rodents, postnatal myelination begins as early as postnatal day 7 (P7). It peaks at P15–21 in the corpus callosum and cerebellar white matter and is mostly completed by P60 but varies in rates depending on the neuroanatomic region [13–15]. In humans, development of myelin extends for much longer peaking at 3–5 years of life with increases in white matter that continue throughout the remainder of childhood and adolescence and do not end until the age of 20 [16, 17].

Initiation of CNS myelination occurs several days after PNS myelination when the diameter of the target axons are approximately 1 μm [13]. The flattened processes of the mature OLs which reside in close proximity of the neurons first extend toward the axons and form troughs around the segment [9, 13]. Extension of the processes causes wrapping of the inner tongue of the sheet in a spiral fashion around the axon. Almost immediately, myelin compaction occurs. At the compaction stage, most of the cytoplasm is expelled, and the cell membranes become set against each other making several specialized domains [2]. Some cytoplasm remains to allow the transport of cellular components in areas rich in microtubules. Due to compaction, the distance between the cytoplasmic surfaces in the compacted regions form a major dense line which are 10.5–11.5 nm apart in the CNS and 11.5–12 nm in the PNS [13].

Oligodendrocytes: Development and Function

Oligodendrocyte Function

As mentioned above, mature OLs are responsible for CNS myelin formation [1]. As well as being essential for CNS

myelin production, past research has also shown that OLs are essential for the maintenance and survival of axons and neurons [4] and provide trophic support to neurons [18]. Accordingly, when OLs are lost, as occurs in some demyelinating diseases including multiple sclerosis (MS), there is a secondary degeneration of axons [18]. Since 99% of an axon's surface can be covered in myelin, little space is available to allow glucose and other metabolites necessary for cell function to enter the axon [4]. Hence, it is thought that energy may be made available to the axons by OLs through their contact with the myelin sheath. An extracellular membrane channel that is predominantly localized in the myelin sheaths, monocarboxylate transporter 1 (MCT1), is responsible for the transport of lactate, pyruvate, ketone bodies, and protons which are essential for maintaining axonal integrity. Lack of MCT1 expression in OLs decreases availability of metabolic energy and therefore affects an energy-dependent process like OL and axonal transport, as seen by the altered morphology of the axons. OLs act as intermediates for metabolite transport which, if inhibited can lead to axonal degeneration and neuronal loss. As well, connections between OL and both astrocytes and neurons may facilitate the transfer of energy metabolites from astrocytes to OL then to neurons.

Development of the Oligodendrocyte Lineage

OLs develop late in CNS development and are among the last cell types to be produced. OLs develop after the initial period of neurogenesis and from the differentiation of neuroepithelial cells that originate from progenitor domains located in forebrain regions (the ganglionic eminences in mice) adjacent to the ventricles and in the developing spinal cord that give rise sequentially to specific neuronal subtypes (motor neurons in the pMN domain of the spinal cord and GABAergic interneurons in the ventral telencephalon), then later to OL progenitor cells [8]. As neurogenesis beginning at embryonic day 9 (E9) in mice progresses from the caudal to rostral regions, neuroepithelial cells first develop into radial glial cells (RGC) and later, to glial cells. RGC have the ability to generate all the neuronal subtypes of the CNS.

Gliogenesis follows the initial period of neurogenesis in the CNS. During this process, distinct progenitor domains in both the spinal cord and forebrain give rise to neurons before switching to the proliferation of OL progenitor cells (OPC), which will migrate and colonize the CNS, and differentiate later into myelinating OLs (Fig. 7.3). While OLs are known to be derived from RGCs, it is unknown whether an intermediate progenitor stage is involved [19, 20]. However, intermediate progenitor cells are the main proliferative cells in the subventricular zone (SVZ) of the embryonic telencephalon which, like RGCs, are also capable of producing neurons or glia cells making it a possible step in OL development.

In both the spinal cord and forebrain, there are three spatiotemporally distinct waves of OPC production and migration [8, 18, 21, 22]. In the forebrain, the first wave of OPC production occurs at about E12.5 in the medial ganglionic eminence (MGE). These OPCs are marked by expression of the homeobox gene *Nkx2.1* [1, 8, 21, 23]. They migrate out from the MGE and colonize the dorsal forebrain. At E15.5, there is a second wave of OPC generation from the lateral ganglionic eminence (LGE), under the control of another homeobox gene, Gsx2 (formerly Gsh2)[21]. A final period of OPC generation occurs perinatally (P0) in an area adjacent to the corpus callosum. These OPCs are marked by another homeobox gene, *Emx1* expression. In the spinal cord, the first wave of OPC differentiation occurs at E12.5 in the ventral neural tube (an area dominated by sonic hedgehog (Shh) signaling), the second at E15.5 in the dorsal neural tube, and the third at birth [8, 22, 24].

Further development of the OPCs into immature OLs occurs during their migration from their main proliferative site. Migration of OPCs from the MGE occurs after their initial proliferation, and by E14.5, they populate the entire telencephalon including the cortex [8]. The postnatally generated OPCs normally seem to replace/outcompete earlier-born ones [18, 21]. For example, a majority of the OPCs in the adult mouse cortex are from the cortical ventricular zone (VZ) with only ~20% from the LGE [25]. After migrating, OLs further differentiate into mature OLs, then into functional, myelinating OLs. As they mature, changes occur to sorting and transport mechanisms in the OL, sending vesicular material into the processes in preparation for myelination [2]. When OLs establish contact with axons, myelin-specific genes are activated, and OLs begin myelination.

During development, most OLs arise from **discrete progenitor domains** that sequentially generate particular neuronal subtypes, followed by OPCs. In the ventral spinal cord, the pMN domain generates motor neurons and OLs. In the ventral telencephalon, the MGE gives rise to both GABAergic interneurons and OPCs [8, 26]. It should be noted that it remains unresolved whether the neuronal subtypes and OLs arise from a common bipotent progenitor cell or from independent populations of neuronal and glial-competent progenitors within each domain [8]. While a common glial progenitor does develop astrocytes and OLs, they generate from largely mutually exclusive regions of the developing CNS [22]. For example, *Olig1/Olig2* double null neural precursor cells are able to generate only astrocytes and no OPCs are specified in the absence of *Olig* gene function [22, 27]. These models and other research indicate that *Olig1* works as a transcriptional regulator for myelin-specific gene expres-

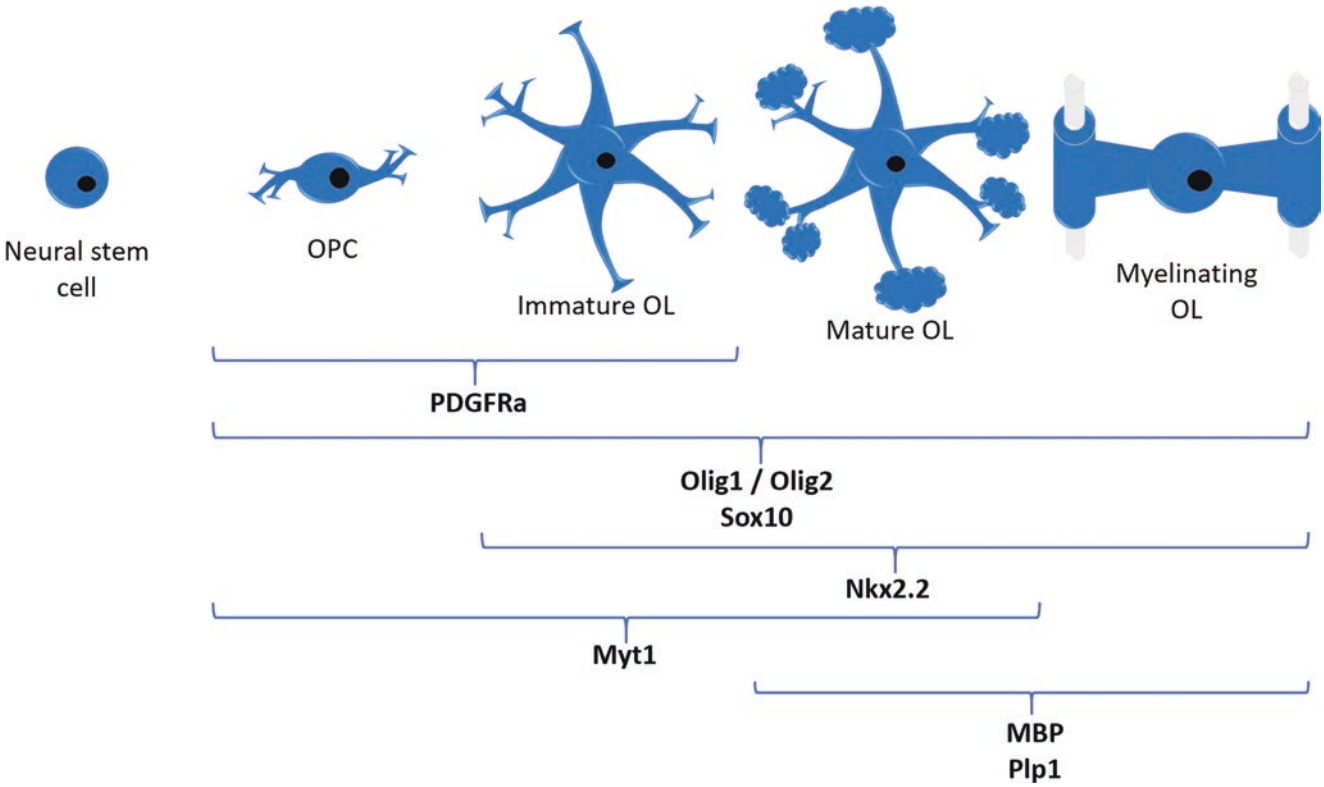

Fig. 7.3 Development of oligodendrocytes and CNS gene expression. There are five overlapping stages of oligodendrocyte development: (1) neural stem cell; (2) OPC; (3) immature oligodendrocytes; (4) mature oligodendrocytes; (5) myelinating oligodendrocytes. Selected gene expression markers are listed for each maturation stage. It is important to note that there is gradient expression of oligodendrocyte and myelin genes throughout oligodendrocyte development. Legend: Platelet-derived growth factor receptor alpha (*PDGFRa*), oligodendrocyte transcription factor 1 (*Olig1*), oligodendrocyte transcription factor 2 (*Olig2*), SRY-box transcription factor (*Sox10*), NK2 homeobox 2 (*Nkx2.2*), myelin transcription factor (*Myt1*), myelin basic protein (*MBP*), and proteolipid protein 1 (*Plp1*)

sion, inducing the expression of OL-genes while consequently repressing astrocyte-specific genes [28].

Multiple intrinsic and extrinsic factors are responsible for establishing the progenitor domains mentioned above, specifying the progenitors that will generate OPCs, and regulating the switch between neuron and OPC production. Development of undifferentiated cells into specific neuronal or glial cells is dependent on the level of signaling factors and the consequently induced genes [8, 22].

Early patterning of progenitor domains involves establishment of spatially restricted expression of various transcription factors (TFs) that act on target genes to determine progenitor cell identity. These TF expression patterns are established very early in development by gradients of morphogens/signaling molecules such as **sonic hedgehog** (Shh) [8, 22]. Shh is the ligand for one of the most important signaling pathways involved in early OL development and the regulation of intrinsic TFs. It establishes oligodendrogenic progenitor domains and induces expression of the *Olig* genes, which are essential for OL specification. Graded Shh signaling in the ventral spinal cord first establishes the pMN domain by regulating expression of the TFs *Nkx6.1*, *Nkx6.2*, and *Olig2* [23]. *Olig2* expression is required for the pMN domain to produce both motor neurons and OPCs; in its absence, this domain instead generates interneurons and astrocytes [22]. Shh signaling continues to be required for OPC specification and development until E12.5. Meanwhile, bone morphogenic protein (BMP) signaling inhibits oligodendrogenesis in the dorsal spinal cord [22, 24]. Shh-regulated *Olig*1/2 expression in the MGE is also involved in forebrain OPC specification [23, 29–31]. In the ventral telencephalon, *Nkx2.1* induces Shh signaling in the MGE which is required for early forebrain oligodendrogenesis. In *Nkx2.1*-null mice, no expression of the OPC marker platelet-derived growth factor receptor alpha (PDGFRa) can be detected in the ventral telencephalon at E14.5 [31, 32]. Shh's importance in early OPC development arises largely via activation of expression of the *Olig1/2* basic helix loop helix (HLH) TFs which are required for OPC specification throughout the CNS, particularly *Olig2*. These genes are discussed later in this chapter in more detail. Subsequent waves of OPCs are also produced in a Shh-independent manner [18]. Later events in OPC development, such as migration, are also Shh-independent [8].

Another signaling pathway that may contribute to the development of OLs is the **Notch-Delta pathway**, which also interacts with the Shh pathway [23, 33, 34]. An increase in Notch signaling leads to excess amounts of OPCs, while OLs fail to form when Notch signaling is inhibited [35]. However, the Notch pathway seems to act in a permissive as opposed to an instructive role with respect to cell fate determination since the timing for OPC production is unchanged even with increased signaling. The importance of Notch pathway activity in OL development may be to maintain sufficient numbers of undifferentiated progenitors that will produce glia after the initial period of neurogenesis. For example, at least in the spinal cord pMN domain, continued Notch-Delta activity appears necessary until the onset of OL production to maintain the pool of glial-competent progenitors [22], and in *Notch* mutant mice, the pMN domain generates only motor neurons. Other signaling pathways that have been implicated in myelin regulation are Fyn kinase and focal adhesion kinase (FAK) signaling which integrates extracellular matrix (ECM) signals and axonal signals to induce cytoskeletal changes, differentiation, and promotion of morphological maturation [5]. Downstream of Fyn and FAK is the larger Akt/mTOR (mammalian target of rapamycin) pathway that is both necessary and sufficient to regulate myelination.

Oligodendrocytes in the Adult CNS

Throughout adult life, some undifferentiated OPCs remain in the adult CNS as the main proliferating cells [36]. These groups of OPCs are considered adult stem cells and proliferate throughout life allowing for some capacity of self-renewal and remyelination. Neural stem cells in the SVZ in the adult CNS produce OPCs and also contribute to remyelination in the rodent SVZ area; however, this may not be completely similar to the human SVZ [24]. The adult OPCs react to demyelination and lesions by accelerating their cell cycle and increasing OL production [23]. Retention of their progenitor characteristics such as proliferation and migration aids in OL roles in the adult CNS.

The number of OLs in the human adult white matter is established in childhood and remains stable throughout adulthood [17]. This may be due to OL exchange potentially limiting neurological functions since it has been observed that local demyelination limits signal transmission during the clearance of old OLs and the formation of new OLs. During learning, there is an increase in white matter; however, new OLs are not responsible for this change as the number of newly developed OLs cannot account for the total white matter increase. However, it is possible that mature OLs can establish the thickness of the myelin sheaths.

Genes Implicated in Oligodendrocyte Development

Several genes are expressed during the specification to maturation processes of OL development. These include *Olig1, Olig2, PDGFRa, Sox10, Nkx2.2, MBP, Myt1, Cnp,* and *Plp1* (Fig. 7.3) [4, 33, 34]. These genes are controlled by several TFs which are considered intrinsic signals, in contrast to external signals such as growth factors, and even elements from the ECM [5]. During the time constrained period of gene activation, gene markers for OPCs are *Sox10* and *PDGFRa,* while mature OLs express MBP and PLP1 protein [8]. Several monoclonal antibodies such as O3 and O4, which react with glycolipids that make up the OL cell surfaces, have also been used as OL specific markers [37].

Olig Genes

Olig1 and *Olig2* encode the genes oligodendrocyte transcription factors 1 and 2, respectively. Together with the related gene *Olig3*, which does not function in oligodendrocyte development, they form a distinct subclass of bHLH TFs. *Olig1* and *Olig2* were first identified based on their prominent expression in both myelinating Ols and OPC ([38, 39]; Q. [40]), and they play critical roles in multiple facets of OL lineage development.

Olig1 and *Olig2* are expressed in both OPCs and myelinating Ols and are known to promote OL differentiation and remyelination in the mouse CNS ([1, 38, 41, 42]; Q. [40]). *Olig1* is responsible for the maintenance of myelin sheaths and axonal integrity during brain development [1]. However, unlike *Olig2* which is necessary for OPC specification in the mouse spinal cord, *Olig1* has been shown to be non-essential for OL development and is unnecessary for spinal cord OPC specification as long as *Olig2* is expressed [43]. In mice, *Olig1* expression is the earliest marker for spinal cord OPCs and in the mature CNS it is only expressed in OLs. Although *Olig1* mRNA is present before E11, it is not translated into OLIG1 until E18.5 supporting that *Olig1* is not necessary for specification [44]. However, in the developing embryo, it may have roles in the formation of other neural lineages [38]. Its expression persists in mature OLs possibly pointing to its role in OL survival, proliferation, and maturation. In the forebrain, the homeodomain transcription factors DLX1 and DLX2 negatively regulate OPC formation by transcriptional repression of *Olig2* and other genes to favor development of GABAergic interneurons rather than oligodendrocytes from common progenitor cells [26]. Similarly, *Olig1* suppresses *Dlx* genes [34].

The importance of the *Olig* genes is apparent when studying the *Olig1/2* double knock-out (DKO) mice. In the absence of both *Olig* genes, OLs fail to develop (Q. [45]). Progenitor cells expressing *Olig* genes normally regulate the

development of motoneurons and Ols; however, the DKO develops interneurons and astrocytes. The complete failure to produce Ols in the DKO show that *Olig* genes are required for all Ols.

Plp1

Out of the structural proteins that make up myelin, PLP1 and its spliced isoform DM20 are the most abundant, with PLP1 being more prominent making up ~50% of the CNS myelin [2, 4, 46, 47]. *Plp1* encodes for the PLP1 protein which is a hydrophobic integral transmembrane protein that can bind to fatty acids with several cysteine residues [1, 46, 47]. Specifically, in the CNS, PLP1 is thought to have a role in maintaining the structure of the myelin sheath by cementing together leaflets of myelin. It also has roles in mRNA, protein transport, OL cell death regulation, and OPC development due to its expression prior to myelin formation [46–48]. In the PNS, PLP1 expression is not prominent in Schwann cells and it may not have a prominent role [1].

Several factors such as Shh in the spinal cord and TFs OLIG1 and OLIG2 in the forebrain are able to affect *Plp1* expression [1]. From *Plp1*, the alternatively spliced and smaller DM20 protein is also produced; however, the ratio of PLP1 to DM20 is normally in favor of PLP1 [2, 4, 28]. Should DM20 levels exceed PLP1, rodents develop abnormal myelin structure and myelin instability with myelin membrane degeneration in rats. Mutations to *Plp1* usually results in hypomyelination as well as degeneration of OLs [2].

Mbp

This gene is located on chromosome 18 and encodes for the prominent myelin protein myelin basic protein (MBP) which is essential for CNS compact myelin production [49]. *Mbp* expression can be detected as early as 2 days postnatally and peaks at 16 to 20 days [12]. The hydrophilic, extrinsic membrane protein MBP is one of the products of the larger gene complex, Golli (Genes of OL Lineage), which includes the seven exons required for the classic MBP [6, 12, 50]. This gene also has several isoforms from alternative splicing of a single mRNA transcript with the major MBP isoform being the human 18.5 kDa and 14 kDa in rodents [2, 6]. It is involved in myelin compaction and has been suggested in having a role in determining the structure of CNS myelin [2, 51]. Without *Mbp*, there is no formation of the major dense line suggesting that MBP is involved in adhesion of the cytosolic surfaces and is known to be present in the compact internodal myelin [6, 12, 52]. MBP may also have roles in signaling, cytoskeletal interactions, and regulation of other CNS myelin and OL genes [6]. *MBP* has differential expression during OL development and its loss can result in severe dysmyelination [6, 12, 50, 52].

Cnp

Another OL gene is *Cnp1* which encodes for the 2′,3′-cyclic nucleotide phosphodiesterase shown to have high CNS expression and associated with myelination [4, 12]. CNP forms 4% of the myelin proteins along with its two isoforms which derive from an alternatively spliced mRNA. Its expression is noted in mature CNS OLs and is used as a common marker for the myelin producing cells [53]. Mice that lack *Cnp1* have visibly unaffected myelin; however, axonal loss and the consequent development of a severe neurodegenerative disorder with premature death before 1 year of life was observed. Hence, while CNP does not play a role in the physical stability of myelin, it does play a part in OLs' role in axonal support and CNS function.

PDGFRa

Tyrosine kinase alpha-receptors for platelet-derived growth factor (PDGFRa) are expressed in the forebrain at a timepoint before the appearance of either neurons or glia [2, 54, 55]. In the spinal cord, OPCs are identifiable due to their expression of PDGFRa. PDGFR are mitogenic and chemotactic for progenitor cells, and has been implicated in OPC proliferation and short-term survival, but not in specification since overexpression only results in an increase in OL numbers [2, 56, 57]. Expression of *PDGFRa* at E12.5–13.5 in the mouse was previously used as a marker for OPCs in the spinal cord; however, *Olig* genes were reported to be expressed at least 72 hours before and some reports stated that *Olig2* is expressed 1–1.5 days while *Olig1* is detected 1 day earlier ([38, 58]; Q. [40]). It is interesting to note that the three genes overlap in the VZ and are expressed in the same cells [38].

Mouse Models

Olig1-Null Mice

Production of *Olig1*-null mice demonstrates that the gene is necessary for the commencement of OL myelinogenesis since *Olig1*-null mice display severe deficits as well as premature death at P14 [1]. These defects include severe hypomyelination, CNS axonal swelling, axonal degeneration, and gliosis, the reaction of glial cells to CNS damage. These malformations and defects lead to neurological deficits such as abnormal limb clasping, generalized tremor, tonic seizures, and ataxia which are more severe than other myelin-gene mutations that produce relatively normal myelin sheaths. Such deficits are indicative of myelin-deficient animals with interrupted myelin sheath production but normal OL differentiation. In contrast, heterozygous *Olig1*-mice achieve normal lifespans without neurological deficits. Others have

shown that *Olig1*-null mice only have a transient delay in myelination [43].

Expression of genes such as *Mbp* and *Plp1/Dm-20* was not observed in certain brain regions of the *Olig1*-null mice with overall decreased expression of several major myelin genes—*Mbp, Plp1, Mag, Cgt,* and *Cnp* [1]. *Cgt* (or *UGT8* in humans) encodes the UDP-galactose ceramide galactosyltransferase enzyme. Accompanying the decreased gene expression, myelin was undetectable around the axons of tracts spanning the optic nerve and corpus callosum. At an early stage, processes of the OL recognize, extend to and contact the neuronal axon, but are unable to continue with myelinogenesis in the absence of *Olig1* function. There are few mature OLs observed in these null mice indicating that OPCs are capable of migration into white matter tracts and able to initiate differentiation; however, they are incapable of inducing myelin-specific genes. This knockout mouse supports the concept of an inverse relationship between the presence of myelin and the severity of a disease.

Compared to the brain which has essentially no myelin production around regions like the optic nerve, the spinal cord of the *Olig1* null mouse has few myelin sheaths [1]. The myelin is compact and thinner when compared to heterozygous mice. However, when taken together, myelin generation in the spinal cord is less dependent on *Olig1*.

Jimpy

Jimpy mutants carry a sex-linked recessive lethal gene that only affects hemizygous males [59, 60]. A specific point mutation in the X-linked *Plp1* gene results in a deletion of exon 5 [61]. Characteristics of the animal is an intentional tremor that was classified at approximately P11. The tremor is not present during rest but upon movement; a violent tremor that originates from the hindlimbs of affected male mice becomes evident [59]. Presence of weak hind limbs by weaning age and in some cases, complete paralysis of the hind limbs have also been noted. By week 4 of life, there is the appearance of generalized tonic-clonic seizures with durations of less than 1 minute without a focal onset. Postictally, there is complete cessation of action then continuation of tasks prior to onset [59, 60]. Animals have an early death at ~30 days of life normally after a seizure. The CNS of the mutants lack myelin when compared to normal littermates, with no PNS phenotype.

Rumpshaker

Similar to *jimpy,* this mutant has an X-linked inherited defect in *Plp1* [62]. The *rumpshaker* mutation is characterized by generalized hypomyelination in the CNS but without changes to PNS myelination. The mutation is not lethal and manifests as a mild tremor that later disappears. No seizures and normal lifespans are observed. Hemizygous males still have the potential to breed [62, 63]. However, there is lack of PLP1 protein expression regardless of the abundance of mRNA which may result from translational or post-translational abnormalities [62].

Shiverer

Shiverer (*shi/shi*) mouse have an autosomal recessive mutation in the *Mbp* gene located on chromosome 18 with deletions to all (exon 3–7) but the first two exons of the gene resulting in loss of MBP protein expression [9, 49, 51]. Due to MBP's structural roles, the CNS of *shiverer* mice is hypomyelinated; however, the PNS is normal. Phenotypically, these mutants have generalized tremors during movement causing a trembling gait which occurs at about 12 days after birth and lasts until their early death which ranges from 50 to 100 days of life [9, 64]. As the animal ages, there are prominent tremors in their hind limbs and some mice with hind limb paralysis. These tremors are only prominent during movement and are absent during rest. Prior to the appearance of these shivering tremors, these mutants appear phenotypically similar to their normal littermates. After 30 to 60 days, tonic seizures and convulsions begin and consequently occur more frequently and with increased severity. Attacks can be initiated with sound, motion, light, and handling.

Disorders of Myelination

Demyelination is the pathological loss of the myelin sheaths around neuronal axons (R. J. [11]). Demyelination disorders are categorized in two groups: primary versus secondary demyelination (Fig. 7.4). Primary demyelination occurs due to direct damage to OLs in the CNS and/or Schwann cells in the PNS. Secondary demyelination or Wallerian degeneration is myelin loss due to primary axonal loss. In the following paragraphs, the majority of the focus will be on disorders or diseases that are categorized under primary demyelination.

In the occurrence of demyelination, there are several changes that occur to compensate for the loss of myelin and OLs. The loss of the saltatory conduction is usually compensated for by the addition of Na^+ channels along the axon which makes abnormal continuous conduction possible [65]. During pathological conditions, neural progenitor cells situated in the SVZ that are normally committed to becoming neurons are induced to generate OLs through inhibition of specific genes and signaling pathways such as *Gad* (glutamic acid decarboxylase) and BMP [24]. From here, they subse-

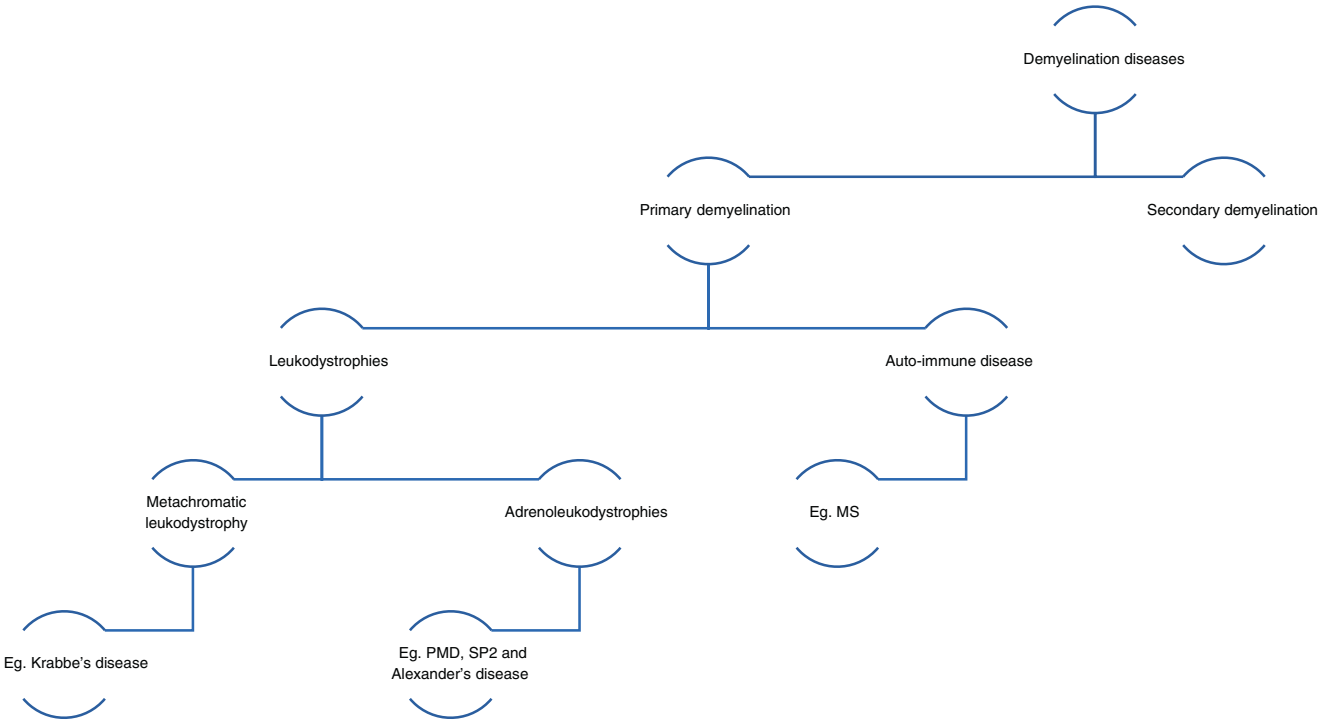

Fig. 7.4 Demyelinating diseases. Demyelinating diseases are divided into primary (caused by damage to oligodendrocytes or myelin) and secondary demyelination (caused by damage to axons). Primary demyelination is divided into leukodystrophies due to oligodendrocyte and myelin gene mutations and autoimmune diseases with myelin damage due to excess immune system activation. Leukodystrophies are divided into metachromatic (lysosomal storage dysfunction) and adrenoleukodystrophies (peroxisomal dysfunction)

quently travel to the white matter to replenish the myelin. However, local OPC pools can also repopulate the demyelinated areas.

Leukodystrophies

One of the major causes of primary demyelination are genetic abnormalities that affect the OLs of the CNS (R. J. [11]). Leukodystrophies are genetic disorders of demyelination normally present during pediatric age with general neurological symptoms (R. J. [11, 66]). These diseases can be further divided into defects in lysosomal storage (metachromatic leukodystrophy or **Krabbe's Disease**) or peroxisomal (adrenoleukodystrophy) function. Adrenoleukodystrophies can result from misfolding of key proteins for myelin (example, **Pelizaeus–Merzbacher Disease**, PMD) or defects from astrocytes that support OLs (example, Alexander's disease with gene mutations of the intermediate filament glial fibrillary acidic protein or GFAP).

PMD is a demyelinating and neurodegenerative CNS disorder caused by mutations to the *PLP1* gene located on the X-chromosome [67]. Characteristics of PMD normally manifest in pediatric age as nystagmus, delayed psychomotor development that progresses to spasticity, ataxia, and a shortened lifespan [46, 47]. Severe PMD is apparent at birth or after a few weeks, while classic PMD may be recognized later in childhood. *PLP1* is an X-linked disorder with sons of female carriers having a 50% chance of inheritance while daughters have a 50% chance of being carriers. A majority of PMD patients contain duplication mutations to Xq22 which contains *PLP1*. This mutation corresponds to a decreased number of OLs and dysmyelination due to overexpression of PLP1. Missense or frameshift mutations have the same phenotype and rarely, whole-gene deletions and chromosomal rearrangements of *PLP1* may occur. Unlike PMD, **spastic paraplegia 2** (SPG2) is a mild X-linked syndrome similarly caused by mutations to *PLP1* which manifests as spastic paraparesis and usually with normal lifespans. The decreased severity of SPG2 has been attributed to the relatively normal presence of DM20 which partially substitutes for the mutated *Plp1*.

Due to the prominent expression of PLP1 in the myelin sheath, it was thought that demyelination or hypomyelination was responsible for axonal degeneration. Since this time, secondary microglial activation and inflammatory responses have also been implicated in numerous diseases with axonal degeneration [68] including PMD [67]. Increased

Plp1 expression in the *Plp1tg* transgenic mouse model (containing at least two extra copies of *Plp1*) was observed to have higher densities of microglial cells in both the white and grey matter of the brain as well as morphological changes such as retraction and thickening of processes [67]. The microglial activation was observed at a time point where no myelination is normally present; therefore, the activation was not attributed to demyelination or hypomyelination. The increased microglia were accompanied by increased mRNA expression of several classic inflammatory factors (TNF-a and IL-6).

Autoimmune Disorders

The other cause of primary demyelination is due to inflammatory damage to myelin and OLs (R. J. [11]). The best example of this and the most prominent demyelinating disease is **multiple sclerosis** (MS) [4, 11]. MS is a chronic inflammatory demyelinating disease of the CNS in which the immune system attacks and damages myelin. The causes of MS are complex and include both genetic and environmental components. There is an association between MS and particular human leukocyte antigen (HLA) haplotypes (the *HLA-DRB1* haplotype is associated with increased risk). MS is also more common at higher latitudes (vitamin D hypothesis). The hallmark of this disease in many patients is the relapsing-remitting pattern of neurological symptoms due to demyelinating episodes and consequent functional recovery with inflammatory recession and remyelination during early stages [4, 5]. As the disease progresses, less myelin repair is possible and there is a visible decline in patients' abilities. Eventually, the disease enters the progressive state where the relapsing-remitting stages are replaced by a progressive deterioration, presumably from axonal damage from continuous demyelination. Some patients have a disease course that is characterized by progressive disease from the outset, without clear relapses or periods of remission. Symptoms of MS can be variable as any part of the CNS may be affected, but commonly include weakness, numbness, and vision loss. While there are immunomodulating agents that are effective during the relapsing-remitting stage, they are usually ineffective during the progressive stage of the disease [5]. In MS, the lack and failure of remyelination results in neurodegeneration and this is frequently exacerbated due to the decrease in remyelination during the aging process [66, 69]. Several reasons for the decreased remyelination have been suggested such as a decrease in OPC density, lag in OPC recruitment due to decreased growth factor sensitivity, or possibly decreased OPC differentiation [70, 71]. It has been proposed that decreased OPC differentiation may be dependent on decreased OPC density in the lesioned areas [72].

Remyelination and Rehabilitation Medicine

In a demyelinating injury, there is loss of OLs and myelin. In the CNS, the default response to injury is remyelination, in which the myelin sheath is restored to demyelinated axons. This is a rare example of a true regenerative process occurring in the adult CNS. It involves the generation of new OLs from adult OPCs, which migrate into the demyelinated area, contact the demyelinated axon, and regenerate the myelin sheath. This section will briefly discuss how remyelination occurs, how it is impacted in disease states such as MS, and therapeutic strategies currently under investigation to promote remyelination in disease.

Remyelination

Remyelination involves the generation of new OLs from adult OPCs, in a process that is broadly similar to developmental myelination. The first step involves **activation** of these adult OPCs, which normally reside (and are quiescent) in the subventricular zone and other specialized niches in the CNS. Activation is essentially a switch from this quiescent state to one in which OPCs are able to differentiate and myelinate, and it occurs in response to a local demyelinating injury (Robin JM [73]). Adult OPCs are not able to differentiate into myelinating OLs until they are activated (R. J. [11, 74]). When OPCs are activated, they undergo morphological changes (hypertrophy) and upregulate expression of genes that are involved in development and differentiation of OLs, such as *OLIG2* and *NKX2.2*. OPC activation is triggered by factors secreted by microglia and reactive astrocytes following CNS injury, and it requires the presence of an inflammatory (i.e., innate immune) response [74]. The degree of OPC activation is proportional to the inflammatory response induced by a demyelinating insult [75]. Remyelination is impaired when the innate immune response is suppressed by corticosteroid treatment [75, 76], or when macrophages are depleted or inhibited pharmacologically Robin [73, 77, 78]. Several chemokines and cytokines that are released in association with the macrophage response to a demyelinating injury have been demonstrated to promote OPC proliferation, differentiation, and remyelination. For example, interleukins 6 and 11 and CXCL2 promote OPC differentiation [79–81]. Another function of reactive macrophages in remyelination is to phagocytose myelin debris within the lesion [74, 75]. This is an important step in remyelination, because myelin debris potently inhibits OPC differentiation [11, 75].

Following activation, local OPCs proliferate and migrate into the demyelinated lesion. This is known as the **recruitment** stage. A number of chemotactic cues are involved in directing OPC migration at this stage, including PDGF,

CXCL1, and several of the class 3 semaphorins [74]. Once in the lesion, OPCs exit the cell cycle and differentiate into OLs. Finally, they contact the demyelinated axons and extend cytoplasmic processes, restoring the myelin sheath.

Remyelination Failure

The process of remyelination takes place efficiently in the healthy CNS. However, in the case of many acquired demyelinating diseases, such as MS, remyelination either fails or becomes less efficient over time. This results in persistent functional deficits and disease progression. Various reasons for remyelination failure have been proposed (R. J. [11, 74, 75]).

Two general factors that are known to impact remyelination efficiency are age and sex. As with all regenerative processes, the efficiency of remyelination declines with age. This is primarily due to age-related decreases in the efficiency of both OPC recruitment and differentiation, and can have a major role in remyelination failure and disease progression over time in diseases like MS that tend to be present for many decades across an individual's lifespan.

The basis for the age effect on OPC recruitment and differentiation is multifactorial but it can be explained, in part, by the effects of aging on the innate immune response. Aging results in an impaired macrophage response that is associated with slower expression of inflammatory mediators and which impairs clearance of myelin debris, and these factors impact OPC activation and differentiation. Older OPCs are also less able to respond to remyelination-promoting growth factors. Additionally, aging is associated with impaired epigenetic regulation of the promoters of differentiation-inhibitory genes in OPCs, a process which is required for OPC differentiation. Older OPCs exhibit a lower inherent capacity for differentiation and are less able to respond to pro-differentiation signals than young OPCs [82]. Sex shows an interaction with age in that remyelination efficiency declines more quickly in males than in females. The reasons for this are unknown [74].

Regeneration/Therapeutic Approaches to Restore Myelin

There are two major therapeutic approaches that are under investigation to restore myelin in demyelinating diseases: by providing an external source of OPCs or other exogenous cells that can myelinate, and promotion of endogenous remyelination. Neither approach is currently in clinical use, but testing in animal models of demyelination is underway.

Transplantation of Exogenous OPCs

For genetic demyelinating and hypomyelinating diseases, where no normal OPCs are available, transplantation of OPCs into the CNS is a potential therapeutic strategy to provide normal myelin. This approach has been partially successful in animal models of PMD and in *shiverer* mice, which lack normal myelin due to mutations in *Mbp*. However, it is not likely to work well in diseases like MS where there are multiple, focal demyelinating lesions throughout the CNS [74]. In this case, local delivery of cells to each lesion poses a logistical challenge. Furthermore, as discussed, remyelination failure in most common demyelinating diseases (e.g., MS) does not appear to result primarily from a lack of OPCs but from problems with recruitment or differentiation of OPCs, some of which might be the result of OPC-extrinsic factors such as a lesion environment that does not permit differentiation (R. J. [11]). In these cases, transplantation of OPCs may be of limited use, because they will still be in an environment that renders them unable to myelinate.

Promotion of Endogenous Remyelination

This approach involves either providing factors that stimulate OPC proliferation, migration, activation, or differentiation, or manipulating the lesion environment to be more amenable to those processes, with the goal of enhancing endogenous remyelination. This option is attractive for disorders like MS that involve acquired demyelination and where endogenous remyelination occurs less efficiently over time. Specific methods to promote endogenous remyelination could include delivery of factors into the lesion that promote OPC differentiation. One approach that has shown some success in early-phase clinical trials is the use of targeted antibody therapy against potential inhibitors of OPC differentiation [83]. For example, the monoclonal antibody Ozanezumab, which targets the myelin-associated neurite outgrowth inhibitor NogoA, a suggested OPC differentiation inhibitor, was shown to improve remyelination in lysolecithin-induced spinal tract demyelination in rats [84].

However, logistical challenges remain before this approach can be implemented in the clinic. As we learned, remyelination can fail due to either failure of OPC recruitment or failure of OPC differentiation. There is currently no way to know which is implicated in a particular patient's disease, but the strategies needed to target the different processes will likely be different. For example, PDGF signaling promotes OPC proliferation and migration but it might inhibit differentiation. To predict which process should be targeted in each individual patient, we need better imaging techniques and biomarker knowledge, as well as a more complete understanding of the reasons for remyelination failure in different diseases.

Conclusion

Knowing the function and development of myelin is necessary for our overall understanding of CNS structure and function; the myelin sheath has an essential function in signal conduction. Many genes are needed for the proper production of OLs and myelin with any deficits leading to numerous demyelinating disorders and diseases. However, increasing our knowledge about myelinogenesis can help to develop future therapeutic approaches that may alleviate these demyelinating disorders.

Multiple Choice Questions

Q1. Which of the following statements is FALSE?
- A. Oligodendrocytes myelinate axons in the central nervous system.
- B. Oligodendrocytes can myelinate more than one axon.
- C. Schwann cells myelinate axons in the peripheral nervous system.
- D. Schwann cells can myelinate more than one axon.
- E. None of the above.

Q2. All of the following are protein components of the myelin sheath EXCEPT:
- A. Glycolipids.
- B. Proteolipid protein (PLP).
- C. Myelin basic protein (MBP).
- D. 2′-3′ -cyclic nucleotide 3′ phosphohydrolase (CNP).
- E. Myelin-associated glycoprotein (MAG).

Q3. Which of the following are the CORRECT spatiotemporal periods of oligodendroglial progenitor cell (OPC) generation in the developing spinal cord?
- A. Embryonic day 12.5 (E12.5), medial ganglionic eminence.
- B. E15.5, lateral ganglionic eminence.
- C. Day of birth (P0), adjacent to the corpus callosum.
- D. E12.5, ventral neural tube.
- E. A, B, and C.

Q4. Which of the following genes is NOT a transcription factor important for oligodendrocyte development?
- A. Nkx2.2.
- B. Sox10.
- C. Olig1.
- D. Myt1.
- E. PDGFRa.

Q5. Which mouse model or human disease is NOT linked to the *Plp1* gene?
- A. Jimpy.
- B. Rumpshaker.
- C. Shiverer.
- D. Plp1tg.
- E. Pelizaeus–Merzbacher disease.

Answers: 1D, 2A, 3E, 4E, 5C

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Epigenetics

8

Helena Biasibetti-Brendler and Michael Steffen Kobor

Abbreviations

5caC	5-carboxylcytosine
5fC	5-formylcytosine
5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
6 mA	N^6-methyladenine
ASD	autism spectrum disorder
BAF	BRG1/BRM-associated factor
BDNF	brain-derived neurotrophic factor
CGIs	CpG islands
CNS	central nervous system
CpG	cytosine-guanine dinucleotide
DNA	deoxyribonucleic acid
DNAhm	DNA hydroxymethylation
DNAm	DNA methylation
DNMT	DNA methyltransferase
esBAF	embryonic stem cell BAF
ESC	embryonic stem cell
FASD	fetal alcohol spectrum disorder
HDAC	histone deacetylase
HPA axis	hypothalamic–pituitary–adrenal axis
lncRNA	long non-coding RNA
m^6A	N^6-methyladenosine
miRNA	microRNA
mRNA	messenger RNA
nBAF	neuronal BAF
ncRNA	noncoding RNA
npBAF	neural progenitor BAF
NPC	neural progenitor cell
NSC	neural stem cell
PRC2	polycomb repressive complex 2
PTM	posttranslational modification
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
TET	Ten-Eleven Translocation

Learning Objectives

By the end of this chapter, readers should be able to do the following:

1. Explain what the term epigenetics means and the molecular machinery involved.
2. Understand how dysregulation of epigenetic mechanisms can lead to disease states and be able to discuss examples.
3. Recognize how epigenetic machinery can be targets for therapeutic agents and discuss examples.
4. Name and discuss important neural and developmental pathways that are regulated by epigenetic factors and provide examples of epigenetic effects on development.
5. Discuss epigenetic mechanisms that bridge environmental stimuli and neurodevelopmental outcomes.

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Highlights

- Epigenetic factors are thought to form the regulatory overlay of the genome, playing crucial roles in the global shaping and maintenance of developmental patterns.
- Epigenetic processes may serve as potential mediators of gene–environment interplay, permitting brain-specific plasticity during key developmental windows.
- The dysregulation of epigenetic mechanisms can often lead to pathological consequences, contributing to a spectrum of disease phenotypes and neurodevelopmental disorders.

Introduction

The epigenome plays a vital role in the activation and implementation of developmental processes. The definition of epigenetics is evolving rapidly and includes mechanisms that affect genome function, encompassing modifications of DNA and its regulatory elements, including chromatin, RNA, and non-coding RNA (Fig. 8.1) [1]. The epigenome responds to environmental factors and is influenced by genetic variation [2–4]. Therefore, it is becoming apparent that epigenetic signatures are prime biological mechanisms for responding to environmental stimuli. The epigenome has many components, but rather than functioning in isolation, the modes of epigenetic modulation interact in a dynamic manner and coordinate the complex biology of neurodevelopment along multiple different developmental outcomes. Thus, forms of epigenetic regulation, such as chromatin structure (Fig. 8.1a), DNA modifications (Fig. 8.1b), RNA modifications (Fig. 8.1c), and noncoding RNAs (Fig. 8.1d), can be both causes and consequences of neurological development [5].

Epigenetic mechanisms help direct the establishment of the developing brain, in a process involving a wide variety of cell types coordinating neural structure and function (Fig. 8.2) [6]. Epigenetic signatures are associated with gene expression profiles of development-related pathways in the primitive brain, ensuring adequate numbers of neurons and glia as well as directing maturation of the brain structure [7]. It is becoming increasingly clear that epigenetics plays important roles in neurodevelopment, and perturbations in the underlying machinery may have long-lasting adverse health effects. For example, epigenetic dysregulation has been suggested to contribute to the pathogenesis of autism spectrum disorder (ASD) and fetal alcohol spectrum disorder (FASD), both of which are developmental disorders caused by a combination of genetic and environmental factors [8, 9]. Epigenetic dysregulation could affect the development of specialized cell types and thus affect brain function later in life.

The following section will introduce each of the epigenetic marks that are known to be important for neurodevelopment (Fig. 8.1). Later, this chapter will discuss how these epigenetic mechanisms participate in developmental processes, focusing on normal mammalian brain development. Then, we explore how perturbations in the epigenetic machinery have long-lasting adverse neurodevelopmental outcomes given that the epigenome is malleable and can serve as a prime mechanism for the biological embedding of environmental exposures.

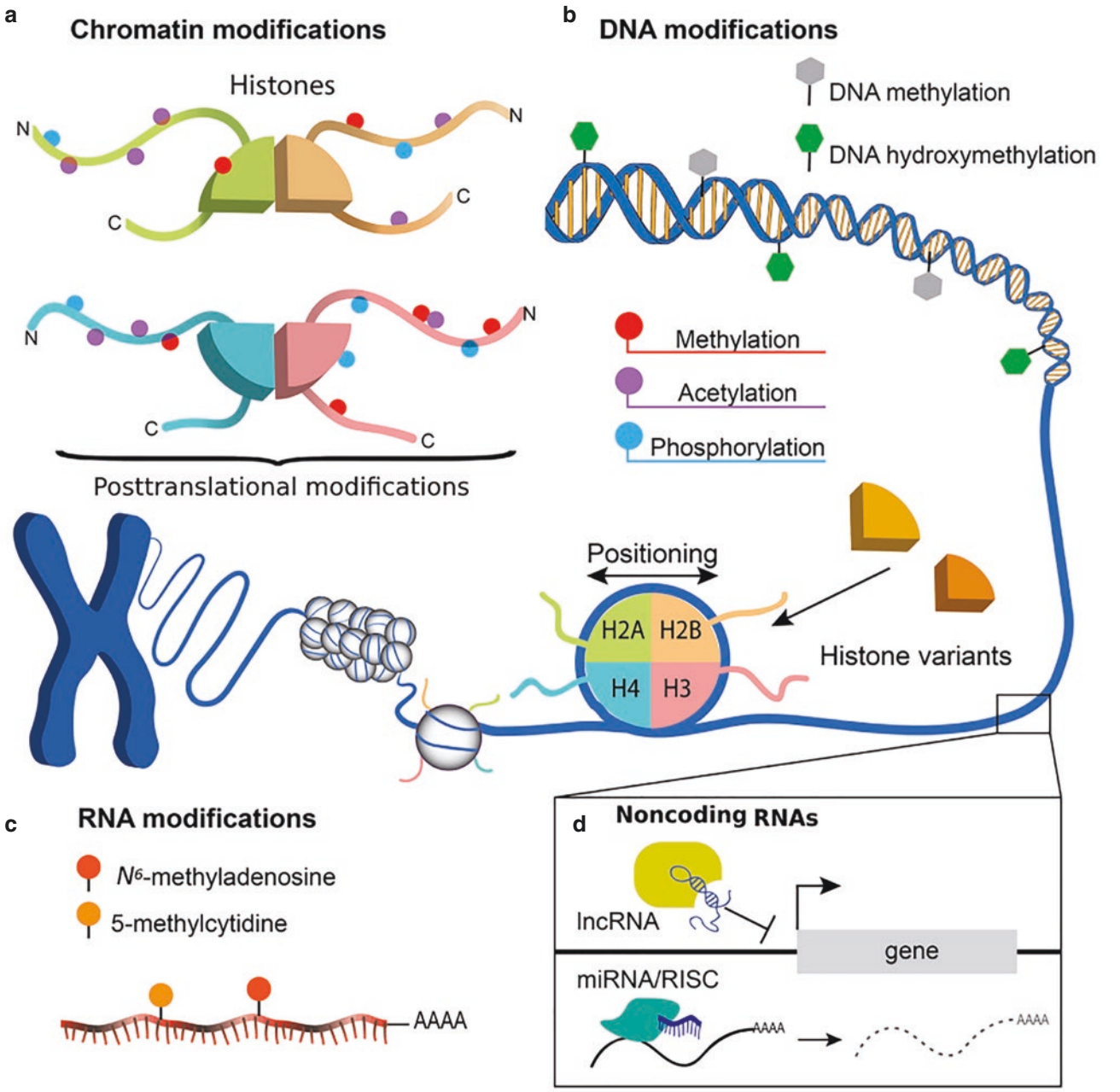

Fig. 8.1 The dynamic epigenetic landscape. (**a**) The nucleosome consists of an octamer of histone proteins tightly bound by DNA. Changes in chromatin structure include posttranslational modification of histones, chromatin remodeling enzymes, and the incorporation of histone variants. (**b**) At the DNA level, covalent addition of a methyl group occurs mainly on the fifth carbon of the cytosine base preceding a guanine (*i.e.*, CpG dinucleotide). DNA methylation (DNAm) and DNA hydroxymethylation (DNAhm) are two of the best studied DNA modifications. (**c**) RNA nucleotides are also subjected to modification by the addition of chemical groups. There are over 160 RNA modifications, including N^6-methyladenosine (m^6A) and 5-methylcytidine (m^5C). (**d**) Noncoding RNAs (ncRNAs), including miRNAs and lncRNAs, are RNA molecules transcribed from DNA but not translated into proteins, which can influence gene expression and neural development by various mechanisms, including direct binding to DNA and RNA interference. Adapted from Aristizabal et al. [2]

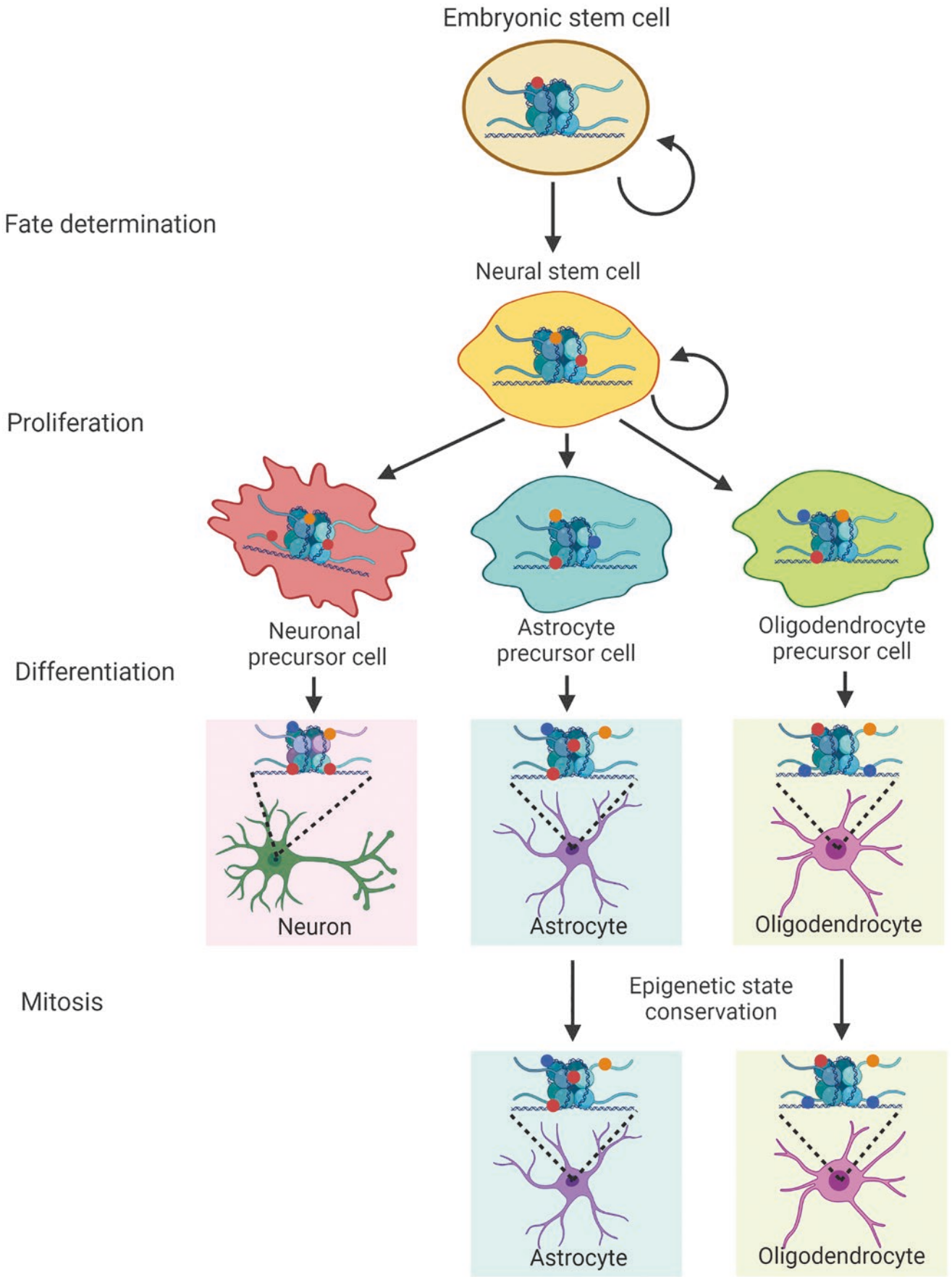

Fig. 8.2 Epigenetic mechanisms involved in cell fate determination. Embryonic stem cells begin with identical genotypes and phenotypes, but extrinsic signals trigger developmental cascades that lead to differentiation. As cells differentiate, their epigenetic profiles become increasingly adapted to their specific functions in the developing organism. Image created with BioRender (https://biorender.com)

Epigenetic Mechanisms

Chromatin and Histones

In eukaryotic cells, DNA does not exist as a naked template, but as a dynamic complex known as chromatin. DNA is wrapped around protein octamers containing two molecules of the canonical histone proteins (H2A, H2B, H3, and H4); these 147-bp units of DNA are also known as nucleosome repeats [10]. Chromatin can exist with multiple degrees of condensation, including euchromatin, a more accessible conformation that is characterized by actively transcribed genes, and heterochromatin, which is highly condensed and tends to contain transcriptionally silenced elements, such as repetitive sequences in pericentromeric and telomeric regions [11]. Protein complexes can modify chromatin structure and regulate access to the underlying DNA sequence. In addition, changes in chromatin condensation influence the accessibility and binding of proteins to chromatin [12]. Chromatin structure is modulated by repositioning of nucleosomes relative to the underlying DNA sequence, the inclusion of histone variants, and histone posttranslational modifications (PTMs) (Fig. 8.1a) [13].

Chromatin Remodeling

Cells have a diverse array of nucleosome remodeling complexes that promote histone sliding, ejection, and histone variant incorporation (Fig. 8.1a). Chromatin remodeling and nucleosome positioning can influence gene expression by either increasing or decreasing the accessibility of a specific region of the DNA, thus affecting the transcriptional machinery and other DNA-binding molecules [14]. These changes in chromatin conformation can impact transcription of genes involved in developmental processes and are intimately related to cell differentiation and neurogenesis. The permissive transcriptional state is characterized by restructuring of the chromatin from a closed to an open conformation, which can be initiated by the binding of chromatin remodelers, such as the BRG1/BRM-associated factor (BAF) complex (mammalian SWI/SNF complex). This open conformation allows transcriptional activators to bind chromatin and influence gene transcription. Changes in the subunit assembly in the BAF complex promote differential nucleosome remodeling and impact gene expression [15].

Histone Variants

Nucleosomes can be modified by replacing canonical histones with histone variants, each of which has a unique structure and function (Fig. 8.1a). Eukaryotes have variants of the canonical histones H2A, H2B, H3, and H4 that are incorporated into chromatin by remodeling enzyme complexes. Histone variants can affect chromatin biology by changing the stability of nucleosomes and recruiting new readers and writers of histone PTMs, thus changing the protein interactome at specific sites [16]. Recent studies have accumulated data on the functional links between histone variants and neurodevelopment [17]. Although the histone variant H3.3 differs from its canonical form by only four amino acids, it is enriched at distinct genomic regions from H3, such as promoters and transcription factor binding sites, and has been shown to participate in neural cell differentiation [18]. A rare mutation in the *SRCAP* (SNF2-related CBP activator protein) gene, which incorporates the H2A.Z variant into chromatin, is a feature of Floating-Harbor syndrome, characterized by intrauterine growth restriction and later intellectual disabilities [19, 20]. Taken together, these observations suggest that epigenetic regulation of histone variants may play important roles in regulating cell differentiation and neurodevelopment.

Histone Posttranslational Modifications

Histone PTMs are characterized by the covalent modification of histone amino acid residues (Fig. 8.1a) and include acetylation, methylation, phosphorylation, sumoylation, ubiquitination, and longer-chain acylations [12]. Although most of these modifications occur on lysine residues, methylation is also found on arginine, and phosphorylation occurs on serine and threonine residues. More than 400 different histone PTMs and thousands of combinations have been identified to date, although most of their biological functions are still unknown [21]. The covalent modifications of histones are reversible, and modulation of histone PTMs requires the activity of several histone-modifying enzymes. Enzymes that add and remove histone PTMs are referred to as "writers" and "erasers," respectively [22]. Histone PTMs influence nucleosome stability and positioning by altering the chemical interactions within nucleosomes, between neighboring nucleosomes, or between histone–DNA contacts. In addition to their roles in influencing histone–DNA and histone–histone interactions, histone PTMs can also be targeted by histone-binding domains or PTM readers, thus mediating fundamental cellular processes, such as gene transcription [23]. Histone PTMs can influence chromatin conformation, promoting the adoption of either an open or a closed state. Lysine acetylation neutralizes the positive charge on histones, thus weakening interactions with negatively charged DNA and promoting a more open chromatin state, while lysine methylation can have either an activating or repressive effect, depending on the position of the amino acid modified [24]. Lysine acetylation and deacetylation are catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [25]. Two of the best

understood lysine methylation marks are methylation of H3K4, which is catalyzed by the SET/MLL complex and tends to permit transcriptional activation, and H3K27, which is catalyzed by the polycomb repressive complex 2 (PRC2) and typically promotes transcriptional repression [26, 27]. The combination of histone PTMs at specific gene regulatory regions and their changes across development culminate in activation or repression of the transcription of target genes.

DNA Modifications

DNA cytosine and adenine nucleotides can be covalently modified by the incorporation of a methyl group, resulting in 5-methylcytosine (5mC) when the methyl group is added to the 5 carbon of cytosine or N^6-methyladenine (6 mA) when the methyl group is added at the amino nitrogen of adenine. 5mC can also be oxidized by enzymes belonging to the Ten-Eleven Translocation (TET) family, forming the derivatives 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). Although there has been research on each of these DNA modifications, this chapter focuses on the best studied types that are known to be integral to neurodevelopmental processes, 5mC and 5hmC, which function primarily in the regulation of gene expression (Fig. 8.1b) [28, 29].

DNA Methylation

DNA methylation (DNAm) of cytosine residues has been well characterized in terms of its distribution and function across the human genome. DNA methyltransferases (DNMTs) add a methyl group to the 5 carbon of cytosines in either the cytosine-guanine (CpG) or non-CpG (CpH) context. The main DNMTs include DNMT1, which is responsible for propagating existing DNAm patterns across cell division, and DNMT3A and DNMT3B, which catalyze de novo DNAm [30, 31]. The general functions of both CpG and non-CpG methylation depend upon genomic location (*e.g.*, at promoters recruiting transcriptional machinery and in the gene body recruiting splicing machinery), and can translate into gene activation or silencing by destabilizing nucleosomes and recruiting proteins that can create a transcriptionally permissive or restrictive chromatin state [30, 32]. Although the number of methylated CpG sites in the genome (80%) is much higher than the number of methylated CpH sites (2%–6%), and both types of cytosine methylation are distributed similarly throughout the genome, non-CpG methylation is enriched in pluripotent stem cells, neurons, and glial cells [32–34]. Methylation is read by methyl-binding proteins, such as methyl-CpG-binding protein 2 (MeCP2) and members of the methyl-CpG-binding domain (MBD) protein family [35]. By binding to methylated CpGs, MeCP2 can act as a transcriptional repressor and recruit other repressive chromatin remodelers, which may lead to a closed chromatin structure [36]. MeCP2 was shown to be important for normal neuronal function and maturation, given its elevated expression levels in these cells and that the neurodevelopmental disorder, Rett syndrome, is caused by a mutation in the gene encoding this protein [37].

DNA Hydroxymethylation

DNAm can be passively lost during cell division or actively removed from cytosines by enzymatic demethylation. The TET family of enzymes is responsible for the active removal of 5mC, which occurs as a multi-step oxidation reaction, generating intermediates, including 5hmC, 5fC, and 5caC, which are oxidized to cytosine [38]. DNA hydroxymethylation (DNAhm) is more than an intermediate in the demethylation process, as it has also been shown to be a stable epigenetic marker that influences transcription [38]. Although DNAhm can recruit chromatin modifiers, *the* full range of mechanisms by which this epigenetic mark can affect gene expression have yet to be completely elucidated [34, 39]. The relative abundance of DNAhm in the brain in comparison to other tissues (five to ten times higher) suggests that this mark could be particularly important for neurological function, and there is a growing body of research assessing the functions of DNAhm in the context of brain development, aging, and neurological diseases. Although the functional mechanisms of action of 5hmC are not yet completely understood, several lines of evidence suggest that DNAhm may be critical for cell viability and normal development, particularly in the brain [33, 35, 39–41].

RNA Modifications

In addition to the impact of DNA modifications and chromatin regulation on transcription, epigenetic mechanisms can act in a posttranscriptional manner, altering mRNA metabolism and coordination of protein synthesis. Similar to DNA and histones, RNA molecules can also be posttranscriptionally modified (Fig. 8.1c) [42]. RNA modifications have impacts on a wide range of biological processes required for normal development in eukaryotes. A wide variety of RNA modifications have emerged, the best studied of which are N^6-methyladenosine (m^6A), 5-methylcytidine (m^5C), and N^1-methyladenosine (m^1A). These modifications influence RNA metabolism, structure, function, stability, and their interactions with other molecules, thus affecting diverse cellular and biological processes and normal tissue development [43].

Noncoding RNAs

The final layer of epigenetic regulation covered in this chapter is mediated by noncoding RNAs (ncRNAs), which are functional RNA molecules that are not translated into protein. ncRNAs encompass several different species of RNA varying in length and function, such as microRNAs (miRNAs, approximately 20 nucleotides in length) and long noncoding RNAs (lncRNAs, greater than 200 residues in length) [44]. ncRNAs play roles in the control of gene expression at the transcriptional and posttranscriptional levels, as well as signaling, RNA processing, and protein synthesis. Since their discovery, the number of known ncRNAs has increased markedly, with approximately 270,000 lncRNA transcripts identified in humans to date [45].

Small Noncoding RNAs

Within the family of small ncRNAs expressed in mammals, which includes small interfering RNAs (siRNAs), tRNA-derived small RNAs (tRFs), and small nuclear RNAs (snRNAs), miRNAs regulate RNA silencing through post-transcriptional regulation of gene expression [46]. miRNAs have been shown to be associated with social and anxiety-related behavior and detrimental social experiences in both humans and animals [47]. miRNAs affect the levels of proteins expressed by their target genes by interacting with proteins to form RNA-induced silencing complexes (RISCs), which degrade mRNAs or inhibit translation [48]. In addition, miRNAs guide chromatin modifications that promote chromosome segregation during cell division and regulate chromosomal and genomic dosage responses [49], and have also been shown to participate in the DNA damage response [50]. In the central nervous system (CNS), miRNAs mediate cell fate specification and plasticity of synaptic connections [51]. Several miRNAs have been implicated in neurodevelopment, with roles in neuronal progenitor cell differentiation/proliferation (*e.g.*, miR-9, miR-124), astrocyte differentiation (*e.g.*, miR-125), and oligodendrocyte differentiation (*e.g.*, miR-138, 145) [52]. Taken together, these observations indicate that miRNAs are important for regulation of gene expression and may be potential regulators of CNS development and neurological function.

Long Noncoding RNAs

The lncRNAs comprise a large family of RNAs 200–100,000 nucleotides in length, with approximately 50,000 in the human genome (Fig. 8.1d). Recent findings have demonstrated the involvement of lncRNAs in gene regulation [53]. The regulatory mechanisms of lncRNAs can be described according to the structures with which they interact (*e.g.*, protein, DNA, or mRNA). lncRNAs can affect protein folding, serve as protein scaffolds, and play roles in enzyme activation. The effects of lncRNAs on chromatin are related to DNA domain looping/folding, transcription, and anchoring. More specifically, lncRNAs can serve as scaffolding for recruitment of histone-modifying enzymes, such as SET1/MLL and PRC2, and contribute to transcriptional activation or suppression [54]. Similarly, lncRNAs and local histone modifiers can interact with DNMTs, either directly or indirectly, to guide DNAm and subsequent transcriptional repression [55]. lncRNAs can also interact directly or indirectly with mRNA and regulate their splicing, degradation, or translation [53, 56].

lncRNAs have been found in many imprinted gene clusters, suggesting that they play important roles in normal mammalian development. Genomic imprinting results in the monoallelic silencing of certain genes based on the parent-of-origin of the allele. Imprinted genes are typically arranged in clusters called imprinting control regions (ICRs) and are epigenetically regulated in a tissue-specific manner [57]. Mutations in lncRNA genes and dysregulation of lncRNA expression have been implicated in several neurological disorders, including Prader-Willi and Angelman syndrome, which are caused by the improper imprinting of paternally or maternally silenced alleles on chromosome 15q11.2–q13.1, respectively. Perturbation of *Ube3a-ATS* expression, a lncRNA in this locus involved in imprinting of the *Ube3a* gene, leads to detrimental dendritic networks, aberrant synaptic connections, and irregular levels of neurotransmitters in mouse models [58]. However, the mechanisms by which lncRNAs influence imprinting are not completely understood [59].

Conclusions

Taken together, the collective functions and crosstalk between DNA, RNA, and histone modifications, chromatin remodeling complexes, histone variants, and ncRNAs regulate chromatin structure and gene expression in a dynamic and responsive manner. These mechanisms facilitate the diverse cell fate determination in the CNS, regulating neurogenesis, gliogenesis, synaptic plasticity, and brain formation, which will be discussed in more detail below [60–62]. They are also potential candidates to gain insights into the biological embedding of environmental and external stimuli, which have key implications for prenatal and postnatal neurological development [2, 4, 63]. Importantly, disruption of the expression and/or activity of the factors involved in epigenetic regulation may also lead to alterations in neural development [64]. These neural developmental alterations can have downstream consequences for brain health and function, particularly if they occur during sensitive developmental windows. The remainder of the chapter will explore the ways in which these epigenetic mechanisms coordinate neurodevelopment and neurological function throughout life.

Epigenetics of Neurodevelopment

Preconception, Fertilization, and Early Embryogenesis

How early does epigenetic regulation play a role in an organism's development? Prior to conception, a variety of exposures, including toxicants, nutrition, drugs, stress, and exercise, affect the epigenetics of gametes [65]. From a paternal perspective, recent human and mouse model studies have shown that epigenetic alterations in sperm can impact reproductive and offspring health [66]. For example, ethanol exposure in a mouse model was shown to result in hypomethylation at the *Bdnf* (brain-derived neurotrophic factor) promoter in spermatozoa, which was maintained in neurons of the ventral tegmental area of offspring as well as being associated with altered expression of sperm small RNAs (tRNA, mitochondrial small RNA, and miRNA) [67, 68]. Whether transgenerational epigenetic inheritance *via* the gametes can occur is still a matter of some debate, but the current view is that DNAm is a suitable candidate to understand the mechanisms underlying this process.

The first round of epigenetic reprogramming by DNA demethylation occurs during the formation of the primordial germ cells (PGCs), which will later differentiate into gametes [69]. One exception to this reprogramming involves differentially methylated regions (DMRs) of imprinted genes, which are preserved throughout embryonic development (Fig. 8.3) [70]. Imprinting can modulate sex-related differences and other epigenetic mechanisms, such as regulation of growth and metabolic pathways that are key for normal mammalian development [70].

The second major round of epigenetic reprogramming occurs in the zygote soon after fertilization, where most of the genome undergoes DNA demethylation and subsequent re-methylation (Fig. 8.3). This reprogramming is underpinned by *de novo* DNAm as well as the dynamic interplay between passive and active DNA demethylation, with active demethylation involving TET3 oxidation and 5hmC generation [71]. In developing embryos, initial global DNA hypomethylation maintains naïve pluripotency [72]. In mouse embryonic stem cells (ESCs), exit from pluripotency and entry into lineage-specific differentiation in the blastocyst stage are associated with concomitant expression of the DNMT1/3 and TET enzymes, promoting the formation of DNAm as well as DNAhm [72, 73]. Homozygous mutation of *Tet3* in mice leads to neonatal lethality, emphasizing the importance of DNAhm in postimplantation embryonic development [74]. Once cell type-specific DNAm patterns are established, they are sustained by DNMT1 throughout the individual's lifetime by symmetrically methylating CpG sites after cell division, ensuring replication of DNAm patterns across cell generations [75]. At this stage, epigenetic inactivation of the X chromosome in females also occurs,

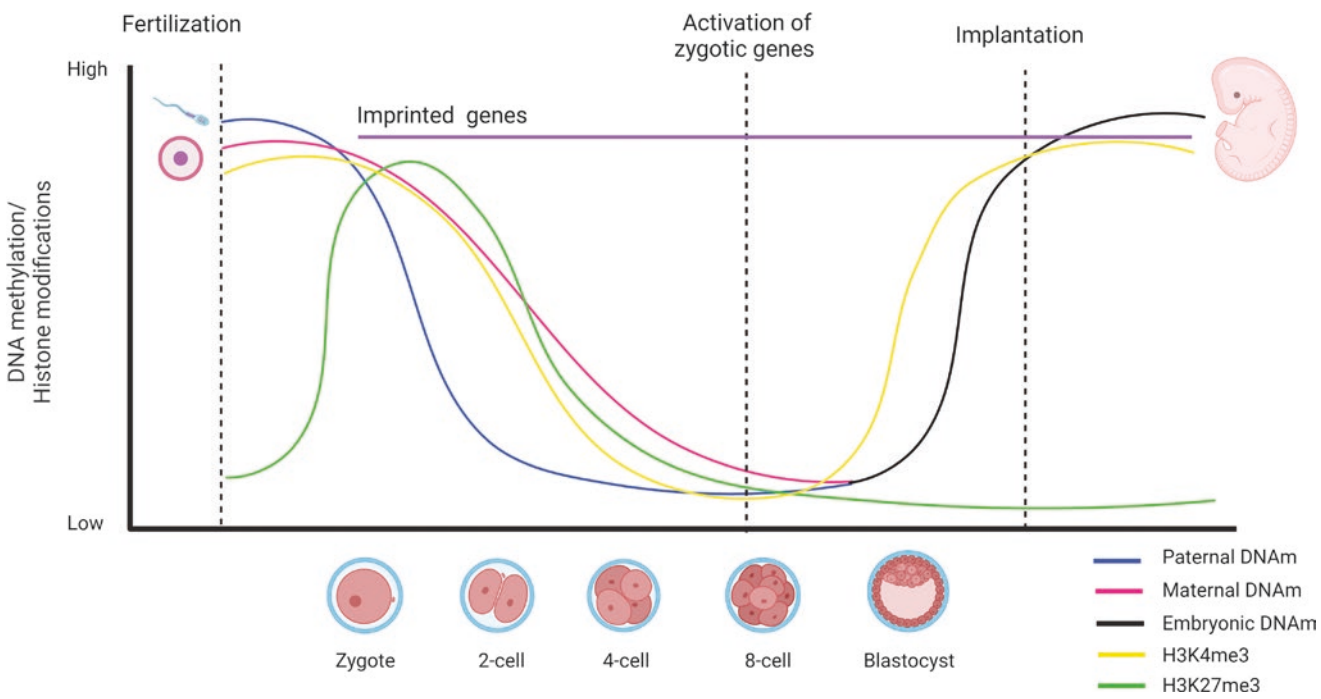

Fig. 8.3 Epigenetic reprogramming in the early embryo. Diagram of major epigenetic marks and their levels from fertilization until embryonic implantation showing dynamic DNAm and histone modifications. Zygotic gene activation represents an important event in initiation of the embryonic epigenetic landscape. Adapted from Smallwood et al. [195]. Image created with BioRender (https://biorender.com)

which accounts for the difference in dosage between males (XY) and females (XX). One copy of the X chromosome is transcriptionally silenced *via* a stochastic process involving DNAm, histone modifications, and ncRNA, leading to tissue heterogeneity in females [76].

Histone PTMs, histone variants, and RNA modifications are also involved in early embryonic development. There is widespread loss of H3K27me3 in promoter regions at the two-cell stage, while H3K4me3 is depleted but is re-established during major zygotic gene activation, which is the period where the embryo takes control over its gene expression that was initially regulated by the maternal genome (Fig. 8.3) [77]. In the blastocyst stage, H3K27me3 is still low but H3K4me3 is re-established, correlating negatively with DNAm levels (Fig. 8.3) [77]. However, H3K27me3 levels increase at specific developmental genes, which are primed to become active by deposition of bivalent H3K4me3/H3K27me3 [77]. Histone variants are also vital to embryonic development, as loss of H3.3, H2A.Z.1, and CENP-A results in embryonic lethality in mouse models before or around the time of gastrulation [78]. Finally, RNA modifications also play roles in mammalian gene expression and development, as removal of the m^6A writer enzyme METTL3 leads to early embryonic lethality in mice [79]. Therefore, epigenetic reprogramming of the developing embryo is highly regulated both temporally and spatially from the germ cell to the blastocyst stage, which is orchestrated by the coordinated interactions of a large number of factors.

Neural Tube Formation and Neurogenesis

Epigenetic mechanisms have been investigated in the context of neural tube formation, or neurulation, which begins around the third week of gestation in humans when the neural plate invaginates to form a tube [80]. DNAm seems to be vital for neural tube formation. *Dnmt3b*$^{-/-}$ mouse embryos show several developmental defects after embryonic day 9.5, including growth impairment and rostral neural tube defects, and inactivation of both Dnmt3a and Dnmt3b causes cranial neural tube defects and early embryonic lethality [81]. Several studies in neural tube defect models showed that histone modifications and nucleosome positioning also play roles in neural tube formation. The HATs GCN5 and CBP/p300, and the H3K27me3 histone demethylase UTX act as transcriptional activators and are required for neural tube closure [82–84]. Thus, several epigenetic regulators were shown to be necessary for neural tube formation and closure.

The neuroectoderm, which gives rise to the nervous system, first emerges when the embryo undergoes gastrulation. Epiblast cells give rise to neuroepithelial cells (NECs), which later generate neural progenitor cells (NPCs) that can differentiate into neurons and glia in the brain [80, 85]. The processes of differentiation from epiblast cells into mature neurons and glia are tightly controlled by epigenetic mechanisms, including DNAm, DNAhm, histone PTMs, chromatin accessibility, and miRNAs. These mechanisms have been primarily studied *in vitro* in ESCs and *in vivo* using mouse models [27, 86–88]. Epigenetic mechanisms are also involved in cell proliferation, which occurs at a high rate during neurogenesis, by orchestrating cell divisions and passing epigenetic signatures through mitotic cell divisions to the daughter cells [89–91]. During neuron production, cytosine modification levels fluctuate and function to repress genes that would have been expressed in alternate lineages (Fig. 8.4) [92]. During neurulation in mice, 5mC is globally deposited in the neuroepithelial layer to prepare NECs for differentiation into NPCs. These 5mC levels decrease as the cells differentiate and migrate away from the ventricle (Fig. 8.4a) [93]. When ESCs differentiate into NPCs in mice, there is a significant increase of DNAm at several gene promoters, including *Oct4*, which regulates pluripotency. This increase in *Oct4* DNAm is associated with loss of *Oct4* expression in differentiated neurons [92]. During this period, PRC2 target sites lose DNAm and become actively expressed [74]. Rapid active DNA demethylation and consequent increases in 5hmC regulate the proliferation of NECs and neurogenesis (Fig. 8.4a) [94]. As brain development progresses, the abundance and genomic distribution of 5hmC undergo marked changes, resulting in the accumulation of 5hmC at neuronal function-related genes during neurogenesis (*e.g.*, synaptic function, dendrite morphogenesis, and axon guidance genes) [74]. The accumulation of 5hmC may function in the demethylation of expressed genes, while retaining MeCP2 occupancy [95]. MeCP2 can act as a transcriptional repressor and recruit other repressive chromatin remodelers, which may lead to a closed chromatin structure [36]. The same process of chromatin compaction that occurs in the differentiation of ESCs to NECs continues during differentiation of NPCs into neurons, as evidenced by increases in the levels of heterochromatin markers [7]. At brain-specific gene promoters, however, gene expression is activated to control differentiation into neurons and glia, as indicated by decreased trimethylation and increased acetylation at histone H3K27 [96]. Non-CpG methylation at CpA sites in human ESCs *in vitro* shows a different pattern to CpG methylation, increasing as differentiation progresses (Fig. 8.4a) [32]. Differentiated neurons have a unique CpA methylation (mCpA) landscape, which may contribute to neural-specific aspects of gene regulation in both development and disease. Once deposited, mCpA is bound by MeCP2 and modulates cell type-specific gene transcription crucial for brain function [7]. Taken together, these observations indicate that the epigenetic machinery is crucial for the

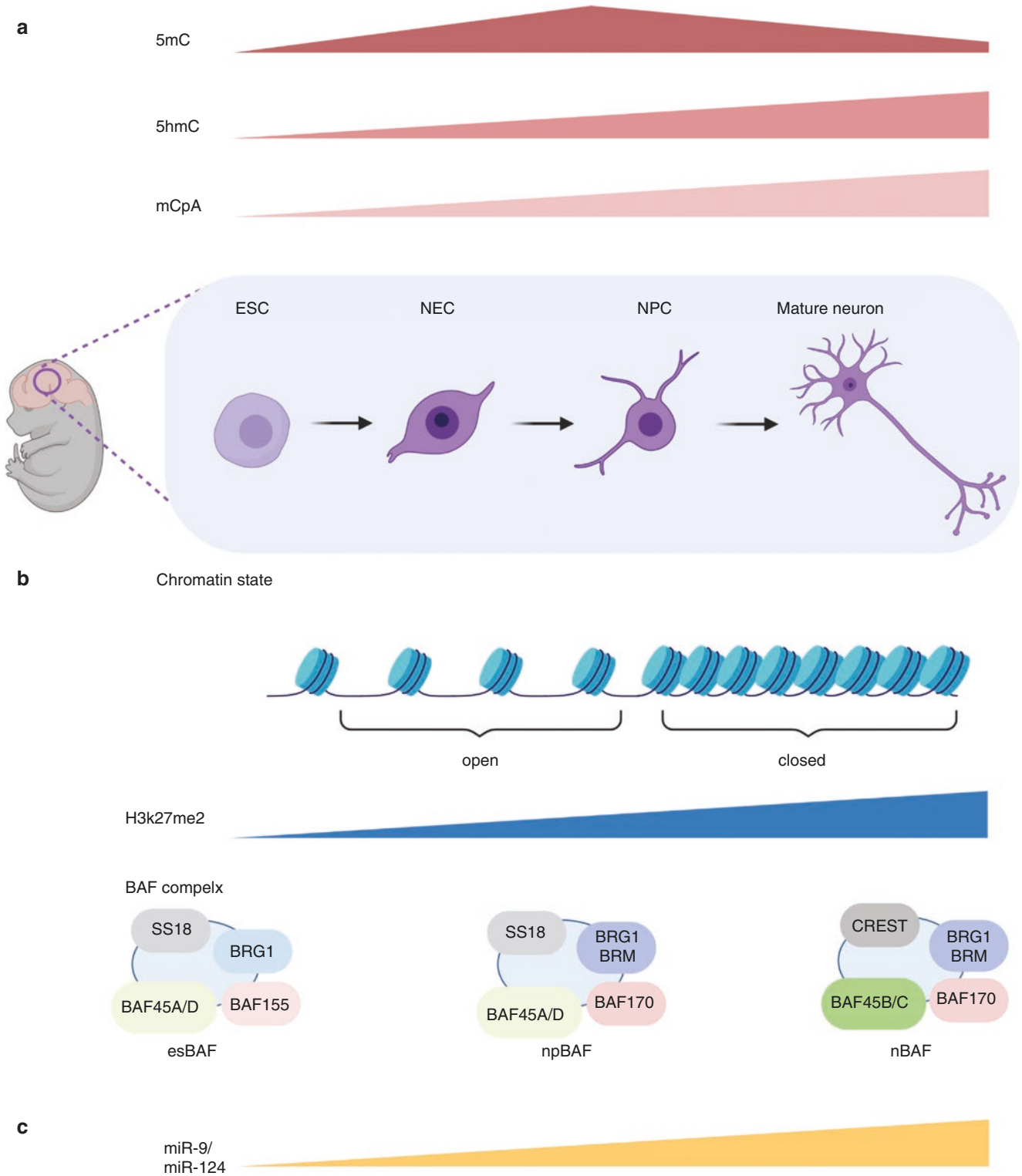

Fig. 8.4 Schematic diagram of global epigenetic landscape trends in neurodevelopment. The colored box in the middle illustrates cell states during neurogenesis from embryonic stem cells (ESCs) to neuroepithelial cells (NECs), then neural progenitor cells (NPCs), and finally mature neurons. (**a**) DNA modification levels are mapped in the bottom panel. Cells gain 5mC at the beginning of cell specification with a subsequent decrease during differentiation and further maturation. Cells also substantially gain 5hmC and mCpA at the beginning of cell differentiation. (**b**) The three-dimensional chromatin structure shifts markedly during neural development. The chromatin is initially in a globally open state and becomes compact in the process of development. H3K27me2 levels increase toward neuron maturation and BAF complex is remodeled during cell differentiation. (**c**) Small noncoding RNAs (miR-9 and miR-124) increase over cell differentiation, peaking in the mature neuron. 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; ESCs, embryonic stem cells; mCpA, CpA methylation; miR-124, microRNA-124; miR-9, microRNA-9; NPCs, neural progenitor cells; NSCs, neural stem cells. Image created with BioRender (https://biorender.com)

formation of neuronal cell types *via* control of the restrictive chromatin state and neural-specific gene regulation to promote the appropriate onset of brain-specific cell differentiation.

Chromatin accessibility is precisely regulated to permit major cell type-specific differential gene expression. *In vitro* analyses of chromatin accessibility showed that the chromatin state is globally open in ESCs and becomes more compact during differentiation into NPCs (Fig. 8.4b) [97, 98], which is partially due to increases in DNAm and H3K27 methylation at targets of PRC2 [99]. In ESCs cultured *in vitro*, approximately 70% of histone H3 is modified to H3K27me2 [99]. This histone mark is linked to facultative heterochromatin and transcriptional repression during early development (Fig. 8.4b) [100]. This period in neurogenesis is also marked by histone turnover and variant exchange. Accumulation of histone variants H2A.X and H3.3 and depletion of H2A.1, H3.1, and H3.2 modulate nucleosome dynamics and promote activity-dependent neuronal transcription [101]. H2A.Z is the only histone variant that remains at a constant level throughout the period of postnatal neurogenesis [102]. However, H2A.Z is also involved in neuronal differentiation, as brain-specific deletion of H2A.Z in mice resulted in enhanced proliferation of NPCs but reduced neuron formation, culminating in defects in cortical neurogenesis [17]. Chromatin is also regulated during the process of cell fate determination by changes in BAF subunits, as pluripotent ESCs develop into NPCs that later differentiate into neurons. Therefore, this complex can be divided into ESC BAF (esBAF), neural progenitor BAF (npBAF), and neuronal BAF (nBAF) (Fig. 8.4b) [103]. For example, knockdown of the *Brg1* or *Baf155* subunits in esBAF can reduce the levels of expression of *Nanog*, *Oct4*, and *Sox2*, which are related to pluripotency [15]. Loss of BAF subunits found in esBAF and npBAF (*e.g.*, Baf45a/d and SS18) results in proliferation defects from ESCs into NSCs [104]. Sokpor et al. [105] summarized the many roles of BAF subunits in neurodevelopment and memory formation.

RNA molecules also help orchestrate the cell differentiation and fate of the two major brain cell types, *i.e.*, neurons and glia. miR-9 and miR-124 are two miRNAs that are highly abundant in the brain and have synergistic effects in controlling neuronal fate. Their levels increase with the onset of neural differentiation and peak in mature neurons (Fig. 8.4c) [52]. Changes in subunits from npBAF to nBAF are mediated by miR-9 and miR-124 and occur with mitotic exit of neural precursors. Premature or delayed expression of nBAF-specific subunits leads to decreases in neuronal proliferation and malformation of dendritic processes [104]. In addition, knockout of *Mettl3* encoding the RNA-modifying enzyme METTL3, in mouse embryos and human ESCs, results in impaired exit from pluripotency, whereas deletion of the m^6A RNA-binding protein YTHDF2 restricts mouse neuronal development *via* reduced cell proliferation and differentiation of NSCs into NPCs [86, 106]. Together, these mechanisms are responsible for transforming chromatin states from permissive to repressive, promoting cell differentiation, and decreasing pluripotency-related factors.

Neurogenesis in the adult brain takes place mainly in the dentate gyrus (DG) in the hippocampus, due to maintenance of NSCs [107]. DNAm intermediates are also observed in the brain throughout life. The hypothalamus has higher levels of 5hmC and lower levels of 5mC than the brainstem, cerebellum, hippocampus, and cerebral cortex in adult mice [108]. In the adult mouse brain, lack of Tet1 leads to weakened hippocampal neurogenesis and poor learning and memory function [109]. Furthermore, DNA demethylation in specific regions of mature neurons enables the expression of brain-related neurotrophins (BDNF, FGF-1, FGF-2), which induce activity-dependent adult neurogenesis [110]. In addition to DNAm intermediates, the adult mouse frontal cortex and human brain show higher levels of non-CpG methylation compared to earlier life stages [111], but the functional significance of this observation is not yet well understood.

Structuring of the Brain Architecture

In the postmitotic phase, immature neurons migrate to various areas of the developing CNS, where they make connections with other neurons with unique structures and functions [85]. Many brain regions are generated with the migration of different neuronal cell types away from the ventricular zone to form the gray matter, including the cortex, thalamus, hippocampus, and cerebellum [85]. In a study comparing epigenetic marks in neurons of the nucleus accumbens, prefrontal cortex, hippocampus, and anterior cingulate gyrus, Rizzardi et al. found widespread differences in CpG methylation across brain regions [112]. The highest levels of non-CpG methylation were found in the context of CpA and CpT. They also showed that the nucleus accumbens had the most unique non-CpG methylation profile [112]. Taken together, these findings illustrate the distinct neuron-specific epigenetic regulation in different brain regions.

The BAF complex is involved in cortex formation, as deletion of this complex from the telencephalon abolished cortex formation [113]. In addition, the loss of neurogenic potential during neocortical development is associated on a large scale with chromatin condensation [114]. During neocortical formation, there is a global increase in the deposition of the active histone mark H3K4me3, accompanied by increases in transcription of major satellite genes [114]. Most lncRNAs have also been shown to be present in specific cell types and different regions of the brain, showing differential expression within the cortical layers in mice [56,

115]. Thus, the formation of specialized neural subtypes, neuronal connections, and the formation of brain regions are correlated with several components of the epigenetic machinery, which can modulate gene expression at the transcriptional and posttranscriptional levels and are involved in brain development as well as neurological diseases.

Epigenetic Regulation of Synapses

The synapses are the points of connection between neurons where chemical and electrical impulse transmission occur [79]. The regulation of synapses is vital for proper brain function, and several epigenetic mechanisms have been shown to play roles in neuronal activity. For example, DNAm was suggested to be involved in synaptic transmission, and the inhibition of DNMT3A in hippocampal neuronal cultures blocks excitatory neurotransmission, leads to decreased synaptic vesicle fusion, and is correlated with increased expression of BDNF, a neurotrophin involved in synaptogenesis, synaptic plasticity, and memory (Fig. 8.5a) [116]. The complex formed between the deacetylase HDAC2 and transcription factor Sp3 facilitates recruitment of HDAC2 to synaptic genes, negatively regulating the promoters of these genes and leading to decreased plasticity, thus resulting in unbalanced growth and reorganization of neural networks (Fig. 8.5b) [117]. Histone turnover is also crucial for neuronal gene transcription and synaptic plasticity. Reducing H3.3 turnover in neurons (Fig. 8.5c) disrupts transcription of activity-dependent synaptic genes, decreases dendritic spines, reduces both excitatory (glutamatergic) and inhibitory (GABAergic) synapses, and impairs cognition [100]. Loss of the RNA m^{6}A modification writer METTL14 is associated with downregulation of synaptic proteins (*i.e.*, reduced synaptic protein levels) (Fig. 8.5d) [118, 119]. These findings suggest that epigenetic mechanisms are instrumental in regulating the transcription of synaptic genes and can influence neuronal communication during development.

Synapse formation is a hallmark of postnatal development, and continues for an extended period after birth and throughout childhood. Synaptic plasticity is one of the major mechanisms linking brain development, learning, and mem-

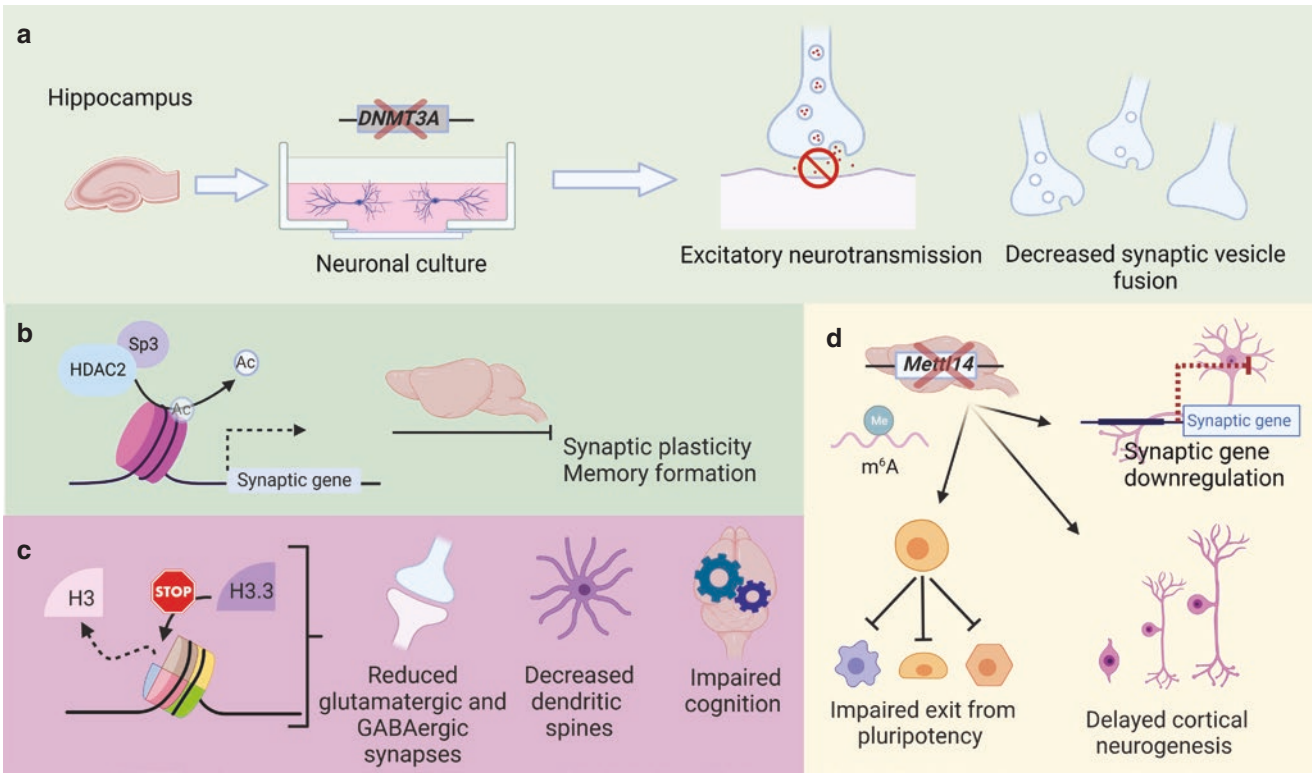

Fig. 8.5 Diverse epigenetic regulation and its mechanisms in synaptic connections. (**a**) Inhibition of DNMT in hippocampal neuronal culture blocks excitatory neurotransmission and leads to decreased synaptic vesicle fusion. (**b**) Complex formation between histone deacetylase HDAC2 and transcription factor Sp3 regulates the promoters of genes implicated in synaptic plasticity and memory formation. (**c**) Histone turnover by reducing H3.3 in neurons has functional effects evidenced by decreases in both glutamatergic and GABAergic synapses, dendritic spine reduction, and impaired cognition. (**d**) Inhibition of methyltransferases that write the RNA modification m^{6}A can be detrimental to neuronal function, *i.e.*, at exit from pluripotency and cortex formation. Image created with BioRender (https://biorender.com)

ory [85, 120]. Memory and learning processes become evident during the first years of childhood. However, although memory may not be clear, early-life events are still biologically imprinted in the epigenome and can shape later life outcomes [2, 121]. Given that the child's brain is sensitive to experiences, the environment plays an important role in development and learning. DNA modifications and chromatin regulation have been shown to be associated with experience-dependent synaptic plasticity and learning outcomes [122].

Balance Between Proliferation and Neuronal Cell Death

CNS development is governed by the balance between cell proliferation and cell death, which is mainly driven by apoptosis. As development proceeds, cell proliferation rates decline, and naturally occurring cell death takes place resulting in the removal of faulty or overabundant neuronal connections [123, 124]. In neuronal injury, histone variants and histone PTMs play roles in programmed cell death. The histone variant H2A.X becomes phosphorylated after DNA damage; this mark recruits the DNA repair machinery and is commonly used to assess the presence of DNA double-strand breaks after exposure to neurotoxins, such as ethanol, in animal and cell culture models of the developing brain [125–128]. Following caspase-mediated apoptosis, the core histone H2B is also phosphorylated on serine 14 as a result of downstream signaling cascades [129]. Cellular sensitivity to death-inducing signals varies markedly at different developmental stages, and epigenetic mechanisms may play roles in maintaining the appropriate cell number through development. In models of FASD, early embryonic and neonatal alcohol exposure were shown to cause neuronal apoptosis and increase histone H2A.X phosphorylation [126, 127]. Thus, neural cell death can occur as a physiological or deleterious event at early stages of neurogenesis, and epigenetic mechanisms contribute to succeeding apoptosis signaling.

Gliogenesis and Myelination

Gliogenesis is the process of glial cell generation (*e.g.*, astrocytes and oligodendrocytes) from glial progenitor cells. Gliogenesis begins with differentiation of NSCs into glial-restricted precursors (GRPs) and oligodendroglial precursors (OPs) [130]. In mice, the catalytic subunit of PRC2, EZH2, is highly expressed and has been suggested to regulate radial glial cell (RGC) identity and proliferation, as well as their transition into glial progenitors. Deletion of *Ezh2*, with the consequent lack of H3K27me3, in RGCs is related to disruption of the timing of differentiation as evidenced by premature astrocyte generation [131]. Repressive H3K9me2/me3 marks, which are established by the methyltransferases SETDB1, SUV39H1, and G9a, also regulate the RGC to glial progenitor cell transition [132]. Similar to *Ezh2*, deletion of *Setdb1* leads to accelerated astrogliogenesis (Fig. 8.6a). With regard to active histone marks, the histone acetylase cAMP-responsive element-binding protein (CREB) acetylates H3K9, H3K14, and H3K27 within target gene promoters, such as *α1-tubulin*, *Gfap*, and *Mbp*, in the cortices in embryonic day 12 to postnatal day 4 mice, peaking in the postnatal stages. *Cbp* knockdown decreases the acetylation levels of the aforementioned promoter regions leading to decreased production of late-born upper-layer neurons and diminished transition of RGCs to glial progenitors [133]. DNAm is also involved in the control of gliogenesis, and most studies have shown that it plays a role in astrocyte differentiation *via* regulation of the JAK-STAT signaling pathways [88, 134]. More specifically, NPCs lacking *Dnmt1* showed premature astroglial differentiation and further differential DNAm in *Gfap* and *Stat* promoters at the gliogenic stage (Fig. 8.6b) [134]. Thus, DNAm may be a key mechanism by which JAK-STAT signaling is inhibited, disrupting the timing of gliogenesis. These data support a model in which histone modifiers and DNAm can regulate gliogenesis and are therefore essential for lineage progression control and appropriate timing of glial production.

Myelin is produced by oligodendrocytes in the CNS, and is vital for proper neuronal impulse conduction [135]. Several studies have demonstrated a link between histone modifications and regulation of oligodendrocyte differentiation. *Hdac3* knockout in NPCs leads to unbalanced glial differentiation favoring astrogliogenesis over oligogenesis and myelination defects [136]. Overexpression of *Ezh2* in NSCs showed the opposite effect, with increased proliferation of oligodendrocyte progenitor cells (OPCs) over astroglial specification [137]. Chemical inhibition of HDAC1/2 in OPCs blocks their proliferation and differentiation [138]. Moyon et al. demonstrated growth arrest in proliferating OPCs in the absence of *Dnmt1* and widespread myelination deficiency [139]. Normal myelination can also be disrupted by lack of miR-219, as OPCs fail to differentiate normally *in vitro* [140]. These observations indicated that different epigenetic components are vital for oligodendrocyte cell development and myelinization.

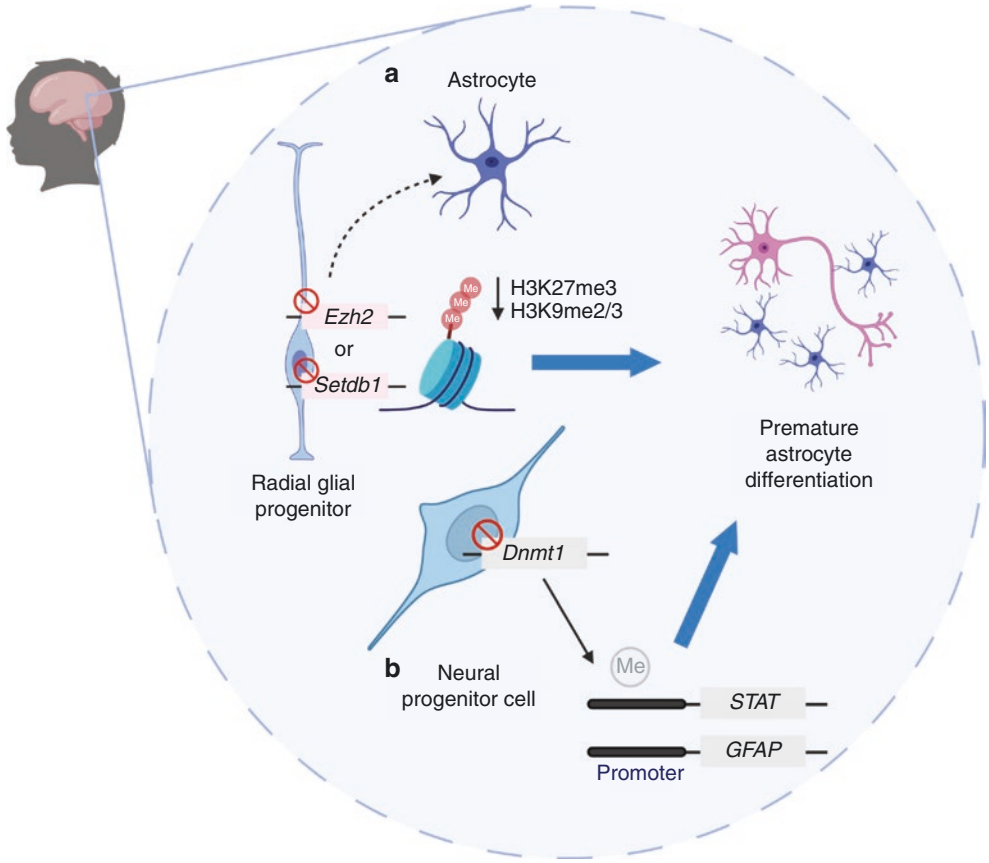

Fig. 8.6 Mechanisms of epigenetic modulation of the timing of glial cell generation. (**a**) Histone methyltransferases have been suggested to regulate RGP identity and proliferation, and RGP to glial progenitor transition. Inhibition of Ezh2 and Setdb1 in RGPs, and thus decreased H3K27 and H3K9 methylation, respectively, are related to premature astrocyte differentiation. (**b**) DNAm is also involved in gliogenesis control, as NPCs lacking *Dnmt1* showed premature astroglial differentiation and further differential DNAm in *Gfap* and *Stat* promoters. Image created with BioRender (https://biorender.com)

Epigenetic Dysregulation and Neurological Disorders

Early epigenetic perturbations predispose the brain toward certain detrimental health outcomes. For example, X chromosome inactivation is extremely important as several nervous system development genes, such as *FMR2* and *MeCP2*, are located on the X chromosome. Mutations of these genes are linked to Fragile X syndrome and Rett syndrome, respectively, both of which present with severe neurological impairments [141]. Mutations in genes encoding proteins involved in transcriptional and chromatin regulation in individuals with ASD may be related to changes in DNAm and histone PTMs [142]. More than 200 differentially expressed lncRNAs were identified in postmortem brain tissue from individuals with ASD, of which 90% were enriched for genomic regions of genes related to neurodevelopment and psychiatric disease [143] (see Part II for more information on ASD). As a teratogen, alcohol exposure can result in an adverse *in utero* environment that leads to numerous detrimental developmental outcomes. Kleiber et al. summarized several prenatal alcohol exposure (PAE) paradigms in critical periods of development and long-term epigenetic dysregulation effects [144] (see Part III for an in-depth report on PAE). Gestation length can also influence the epigenetic profile, as evidenced by multiple DMRs in preterm infants compared to term controls [145]. As preterm labor is the leading identifiable risk factor for cerebral palsy (CP), a small cohort study showed that these DNAm profiles established in childhood persist at least into adolescence [146] (see Part IV for more information on CP). Taken together, several studies showed that dysregulation of the epigenetic machinery is closely related to detrimental neurological outcomes in early life.

Epigenetic dysregulation can also surface in adolescence, which is also marked by the onset of some neurological disorders, such as juvenile Huntington's disease (JHD), Wilson's disease, and schizophrenia [147, 148]. Neuronal epigenetic status was shown to be compromised in a mouse model of JHD, as demonstrated by transcriptional changes in histone regulatory genes, *i.e.*, *EZH1/2*, *CBP*, and *G9a* [149]. Animal models of Wilson's disease show altered levels of methionine metabolism and *S*-adenosylhomocysteine hydrolase activity, which are essential for the proper methylation status [150]. The pubertal period is also marked by an increase in the incidence of schizophrenia at ages between 13 and 18 years [151]. Analysis in the cortex and blood of patients with schizophrenia revealed elevated levels of H3K9me2, an indicator of a restrictive chromatin environment and reduced gene expression, which may be an epigen-

etic mark involved in the etiology of this disease [151]. These data support the correlation of adolescent-onset neurological disorders with epigenetic alterations in adulthood, thus highlighting the importance of examining epigenetic trajectories of neurodevelopment.

Epigenetic Embedding of EarlyLife Influences

Accumulating evidence in both rodent models and humans suggests that the influence of the environment on development can begin *in utero*, which may lead to long-term physiological and behavioral consequences. The developing brain continues to be susceptible to epigenetic reprogramming by environmental exposures in the early postnatal period [3, 63]. Among other factors, the vulnerability of the developing brain to insults depends on the developmental timing of the exposure [152]. Due to the extensive epigenetic machinery modulating cellular differentiation and brain formation, early life is a window in development with major risks of detrimental neurological outcomes, including hypothalamic–pituitary–adrenal (HPA) axis perturbations, neurological impairments, and behavioral disorders (Fig. 8.7) [153, 154]. Therefore, early-life experiences can have a persistent impact on the brain and modulate behavioral and neurodevelopmental outcomes.

Early-life environmental stimuli include maternal nutritional intake, stress, and exposure to teratogens, such as nicotine and ethanol. Exposure to ethanol has been shown

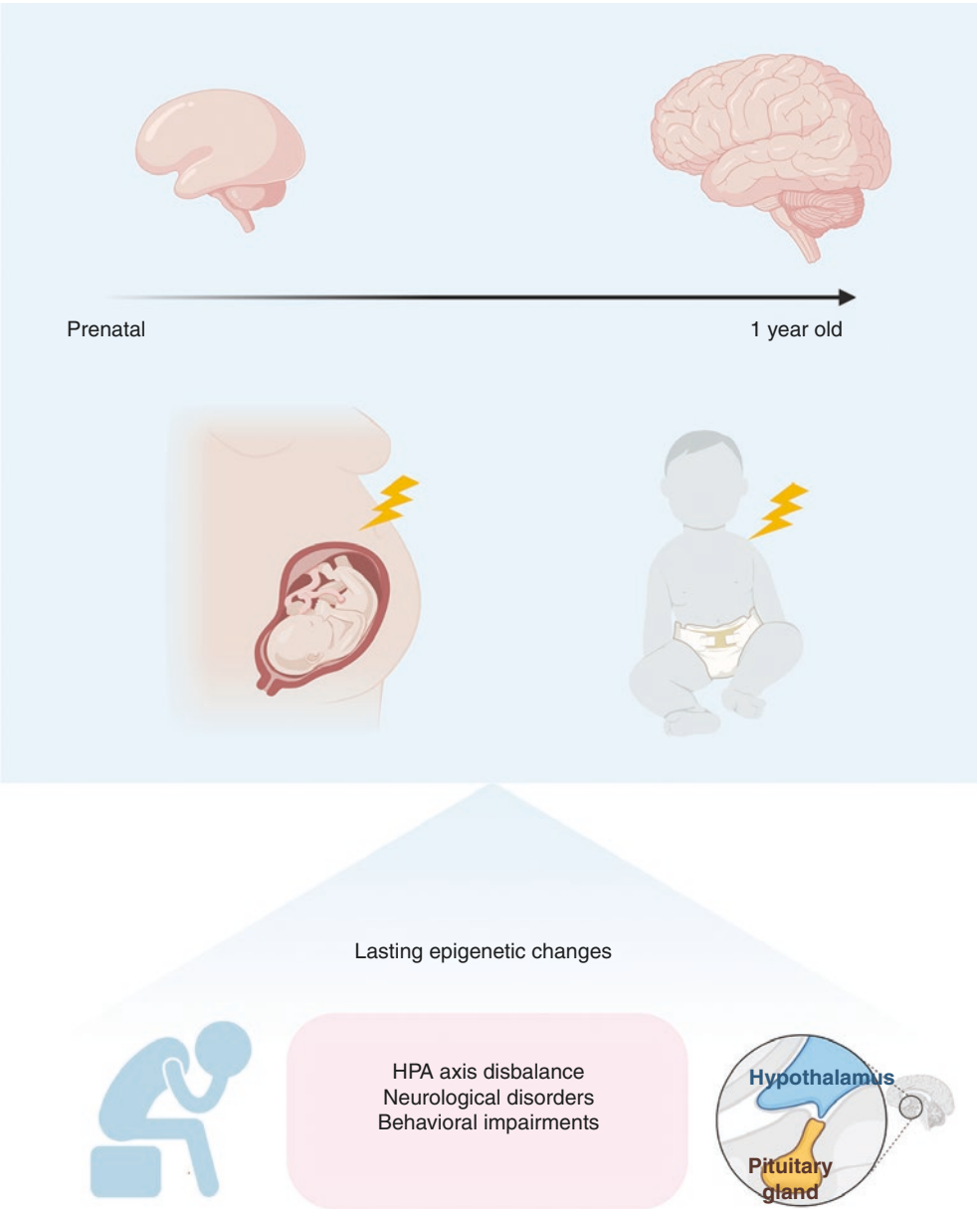

Fig. 8.7 The epigenome is shaped by environmental factors, which can influence phenotypic outcomes across developmental stages. The epigenome can be altered during sensitive periods of heightened plasticity (intrauterine and postnatal periods) resulting in increased risk of complex multifactorial conditions later in life. Adverse experiences and environmental conditions in early life appear to be associated with multiple DNA methylation changes in candidate genes associated with the HPA axis, in addition to anxiety-like and social behavior-related genes across several brain regions. HPA, hypothalamic–pituitary–adrenal. Image created with BioRender (https://biorender.com)

to alter DNAm profiles and chromatin structure in the brain and peripheral tissues in experiments in mouse models and in clinical cohorts [128, 155]. In addition to substance exposure in early life, social interactions and familial environments can also interfere with brain development [156]. Early-life stress *via* maternal behavior, assessed by licking and grooming of mouse pups, was shown to be associated with long-lasting increases in DNAm in the promoter region of the glucocorticoid receptor gene (*Nr3c1*) in the hippocampus into adulthood [157]. In a maternal separation stress model where the pups had limited maternal contact from postnatal days 0 to 10, Murgatroyd et al. demonstrated alterations in gene expression and epigenetic changes in HPA axis-associated genes, characterized by decreased DNAm and MeCP2 binding in their regulatory regions [158]. In addition to stress-related pathways, early-life social disruption epigenetically regulates neurodevelopmental gene targets, as demonstrated by altered hippocampal H3K27me3 at the *N*-methyl-D-aspartate receptor (*Grin2b*) gene [159] and a biphasic response of H3K9me2 in the *Bdnf* gene (*i.e.*, decreased in pups and increased in adulthood), both of which are related to learning and memory [160]. Dysfunctional maternal behavior was also shown to be related to long-lasting changes in *Bdnf* DNAm and altered gene expression in the adult prefrontal cortex in mice [161]. These studies highlight the importance of substance intake and adverse conditions during early development and how they can increase risks of developing neurological disorders later in life.

The responsiveness of epigenetics to environmental stimuli provides insight into long-lasting outcomes in the context of early-life stress [156, 162]. Small regulatory ncRNAs can have enduring roles in the integration of environmental cues with genomic regulation. One specific miRNA, miR-125b-1-3p, has been shown to be associated with the long-term effects of prenatal stress in the hippocampi of rats [163]. miRNAs that are differentially expressed under conditions of stress can increase susceptibility to the development of stress-related disorders later in life [163]. In a preliminary cohort study, postmortem analyses of the hippocampi from suicide victims with a history of childhood abuse were compared to those from controls and suicide victims with no such history of childhood abuse, and the results indicated increased DNAm of cytosine residues in the promoter of the neuron-specific glucocorticoid receptor gene *NR3C1* [164]. Studies leveraging surrogate tissues, *i.e.*, lymphocytes and saliva, showed that exposure to early-life adversity results in increased DNAm in the *CRH* and *POMC* genes, both of which are involved in the HPA axis [156, 165]. DNAm is also involved in positive environmental cues, as offspring of mouse dams housed in enriched environments had DNAm changes in genes associated with HPA axis activity, as well as anxiety-like and social behavior-related genes across several brain regions [166]. Thus, the HPA axis may be epigenetically reprogrammed in the brain due to adverse childhood experiences (*e.g.*, childhood maltreatment, exposure to violence, and parental loss) and beneficial environmental cues. Further studies involving longitudinal epigenetic analysis across childhood development leveraging standardized methods to evaluate epigenetic markers are needed to define healthy neurodevelopmental epigenetic profiles and uncover possible long-lasting biomarkers of adversity in childhood.

Tools and Techniques to Study Epigenetics

Model Organisms and Human Populations

Studies in model organisms have been instrumental in providing insights into many biological processes and fundamental mechanisms underlying disease. Genetics and epigenetics tools that are often conceived and developed in model organisms provide insights into complex aspects of biology, such as disease and behavior [167]. Such studies in model organisms are unlikely to diminish in importance, given the progress in techniques to manipulate the genome *in situ,* which can mimic the endogenous biological landscape and further contribute to our understanding of phenotypic regulation. Epigenetic transgenerational inheritance is also an issue of great clinical interest as environmental outcomes may be carried through the germline, and animal models can yield multiple generations in a short period of time [168]. Animal models are a major asset to epigenetics studies due to the ability to control for confounders and isolate the effects of a specific gene or environmental factor of interest. Rigorous genetic/environmental studies of model organisms will be necessary to gain a deeper understanding of the epigenome. On the other hand, animal models do not accurately represent human physiology, highlighting the importance of human population studies.

Aside from epigenetic characterization of diseases, human population studies have become a vital tool to understand epigenetic signatures in the context of ancestry, ethnicity, social interactions, and psychological phenotypes [29, 169, 170]. Longitudinal human cohorts can provide valuable information on how epigenetic signatures change with time and how they can be associated with different external influences and the biology of disease [2, 171]. A great deal can be learned from epigenetic population studies, as they provide candidate markers for the study of diagnostic and prognostic tools for drug treatments and responses as well as biomarkers for diseases and exposures. However, caution is required when inferring causality from the association between a disease risk genotype and outcomes, as an appropriate experimental design that takes confounding mechanisms into account is required.

DNA Modification Studies

Epigenetic Clocks

DNA methylome research is commonly used to provide insights into the biological underpinnings of environmental exposures and development (see reference [172] for an in-depth comparison of DNAm research methods). Analysis of DNAm is a useful means of predicting epigenetic age, which is correlated with chronological age in healthy individuals [173, 174]. Acceleration or deceleration of epigenetic age, *i.e.*, a deviation above or below one's chronological age, can be calculated using epigenetic clocks. Since the development of the first such epigenetic clock in 2013, several additional clocks have emerged that overcome the initial limitations with regard to tissue specificity and development, including pediatric clocks, phenotypic age clock, placental epigenetic clock, and clocks for animal models [175–178] (see reference [179] for in-depth comparisons of these clocks). Epigenetic age prediction can be applied in developmental biology, cancer, and aging research across a wide range of human tissues and cell types [174]. Changes to epigenetic age have been shown to be correlated with a variety of phenotypes, including developmental trajectories and frailty phenotypes in old age [175, 176]. Applying the original multi-tissue epigenetic clock to the brain indicated that the cerebellum ages more slowly than other brain regions in the same organism [180]. More recently, however, a cortical DNAm age clock was developed, which shows greater accuracy in predicting epigenetic age from brain samples than multi-tissue clocks [181]. Epigenetic clocks have also been used to investigate neurological phenotypes in surrogate tissues. In a small pilot study, McEwen et al. found that children with ASD showed a higher epigenetic age compared to those with typical development, suggesting that the clock has potential for use in screening for ASD [175]. However, the findings from these epigenetic clocks should be interpreted with caution, as the underlying biology behind the CpGs used to predict age is not yet completely understood.

Cell Type Specificity

As the epigenetic machinery is actively involved in development and differentiation of specific cell types, it is not surprising that epigenetic signatures are highly cell type specific, as demonstrated by differences in DNAm profiles between neurons and glial cells [33]. This cell type specificity means that when comparing two samples that have different cell proportions, the differences between the samples will essentially reflect cell type differences rather than any biological condition or phenotype. Therefore, tissue sampling and cell type proportions across individuals should be taken into account to decrease biased interindividual differences [29]. A major caveat in designing epigenetic studies in the CNS in humans is the general inaccessibility of tissue from living individuals. However, the development of new tools has made it possible to interpret DNAm in brain tissue using more readily available surrogates, such as saliva, blood, and buccal epithelial cells. For example, BECon (Blood–Brain Epigenetic Concordance; https://redgar598.shinyapps.io/BECon/) and IMAGE-CpG are web applications that correlate the DNA methylomes of the peripheral tissues and brain to facilitate comparison of cross-tissue DNAm [182, 183]. Such tools facilitate more accurate interpretation of the results of neurological studies performed in surrogate tissues from living individuals.

Chromatin Studies

Chromatin accessibility and regulation studies use methods at both bulk and single-cell levels. Chromatin can be assessed by antibody-based methods (*e.g.*, immunoprecipitation and Western blotting analysis), metabolic labeling, and next-generation sequencing [21, 184]. Technologies that allow the analysis of chromatin modifications on a genome-wide scale provide a comprehensive view of chromatin regulation. However, proposed methods of chromatin analysis have a number of disadvantages due to the presence of heterogeneous mixed cell populations and large cellular sample input [185]. To overcome these disadvantages, genomic techniques have focused on single-cell approaches and the reduction of the necessary sample input and background signals, allowing examination of chromatin structure and transcription factor binding profiles in individual cells with smaller samples (*e.g.*, patient biopsies). Therefore, other advances in small-scale chromatin accessibility measurements are expected to have broad clinical implications, especially for understanding complex diseases (see reference [186] for an in-depth comparison of chromatin regulation research methods).

RNA Studies

High-throughput sequencing technologies have yielded a broader understanding of ncRNAs and RNA modifications, and provided insights into their tissue-specific expression patterns and roles in development. Studies have mapped RNA modifications and their abundance, mostly using antibody immunoprecipitation or chemical treatments and next-generation sequencing [187]. Among the techniques used to characterize RNAs, some focus on localization (*e.g.*, RNA fluorescence *in situ* hybridization), structure (*e.g.*, domain-specific chromatin isolation by RNA purification), functionality (RNA interference), or global analysis (sequencing methods, such as RNA-Seq) [188, 189]. These methods, however, do not distinguish between coding and noncoding transcripts, and therefore, further computational prediction

methods should be used [190]. These techniques can be used to obtain single-molecule transcriptome-wide maps of RNAs, and thus contribute to the identification of putative mechanisms of nervous system development.

Multiomics

As the several layers of the epigenome are interrelated and show complex regulation, an approach that leverages the relationships between the epigenome, proteome, and transcriptome and their spatial profiling in the brain is vital to gain a comprehensive understanding of neurodevelopmental processes and functional outcomes. Several newer techniques focus on multiplex profiling of different omics layers, which can also be done at single-cell resolution [191]. Studies analyzing the DNA methylome, chromatin accessibility, and bound proteins, as well as gene expression by RNA-Seq, can provide useful insights into the biological functions of these epigenetic marks, as not all epigenetic mechanisms influence transcription. Multiomics profiles can be evaluated in a number of ways depending on the categories targeted, such as genome–transcriptome (*e.g.*, TARGET-Seq), genome–transcriptome–DNA methylome (*e.g.*, scTrio-Seq), and transcriptome–DNA methylome–chromatin accessibility (*e.g.*, scNMT-Seq) [192]. These multiomics approaches can allow inference of causality if the experimental design takes into account of genetic and/or temporal data and provide meaningful insights into the spatial and longitudinal patterns of gene expression in the developing brain [191, 193, 194]. Taken together, careful experimental design and detailed profiling of the epigenome, proteome, and transcriptome represent innovative paths of research for the comprehensive study of developmental biology and progression of neurological disorders.

Conclusion

This chapter discussed epigenetic signatures as prime candidates for understanding the lasting effects of dysregulated neuronal development and the influence of external stimuli. Epigenetic studies in both animal models and human populations provide powerful and dynamic tools for understanding developmental progress throughout life, particularly in the context of critical environmental cues and lasting effects on the brain and behavior. In addition to governing embryogenesis, neurogenesis, and neural function, the epigenetic machinery is thought to mediate gene–environment interactions. Despite significant progress in the field of epigenetics, further studies are still needed to evaluate the different layers of epigenetic regulation and their interplay during sensitive developmental windows to gain a comprehensive understanding of their roles in development and disease. Further studies are also needed to clarify the transience or stability of epigenetic marks in neurodevelopmental disorders and the embedding of early environmental inputs. Taken together, the epigenetic landscape has great therapeutic potential in disorders of the CNS and is a promising avenue for the discovery of reliable biomarkers for the diagnosis and treatment of neurodevelopmental disorders.

Multiple Choice Questions

1. What are epigenetic modifications?
   (a) The addition of reversible changes to histone proteins and DNA.
   (b) The removal of nucleosomes from the DNA.
   (c) The addition of more nucleosomes to the DNA.
   (d) Mutation of the DNA sequence.
2. Which of the following are true of epigenetic machinery?
   (a) DNA nucleotides can be covalently modified on cytosine residues.
   (b) Cells utilize ATP-dependent nucleosome remodeling complexes to promote histone sliding, ejection, or the incorporation of histone variants.
   (c) The addition of covalent modifications to histones is reversible.
   (d) All of the above.
3. If epigenetic changes occur within _____ cells, they can be transmitted from one generation to the next.
   (a) Tumor
   (b) Stem
   (c) Neuronal
   (d) Germ line
4. The three-dimensional chromatin structure shifts noticeably during neural development. Chromatin state is globally _____ in the beginning and becomes _____ in the development process.
   (a) Compact; open.
   (b) Open; compact.
   (c) Open; inactive.
   (d) Compact; inactive.
5. Which of the following are true about the effect of environment on epigenetics?
   (a) The concept of Developmental Origins of Health and Disease (DOHaD) postulates that environmental insults in early life do not contribute to long-term risk of disease.
   (b) The influence of the environment on development can only begin after birth.
   (c) Environmental stimuli or exposures during critical or sensitive periods in early life can have lifelong outcomes.
   (d) Early-life environmental stimuli do not include maternal nutritional choices, stress, or exposure to teratogens, such as nicotine and ethanol.

Answers: 1 – (a), 2 – (d), 3 – (d), 4 – (b), 5 – (c)

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The Impact of an Adverse Intrauterine Environment on Neurodevelopment

9

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Abbreviations

AIUI	Ascending Intrauterine Inflammation
AP-1	activator protein 1
BBB	blood–brain barrier
CCL	C-C motif ligand chemokine
CD	cluster of differentiation
CHORIO	chorioamnionitis
CNS	central nervous system
Cox	cyclooxygenase
CP	cerebral palsy
CRP	C-reactive protein
CXCL	C-X-C motif ligand chemokine
CXCR	C-X-C motif chemokine receptor
DAMPS	Damage-Associated Molecular Patterns
FIRS	fetal inflammatory response syndrome
HI	hypoxia-ischemia
HIE	hypoxic ischemic encephalopathy
IFN	gamma- interferon gamma
IL	interleukin
IUGR	Intrauterine growth restriction
IVH	intraventricular hemorrhage
LPS	lipopolysaccharide
MCP-1	monocyte chemoattractant protein-1
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
MPO	myeloperoxidase
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
OPC	oligodendrocyte progenitor cell
P	postnatal
PBI	perinatal brain injury
PBMC	peripheral blood mononuclear cell
PHHP	posthemorrhagic hydrocephalus of prematurity
PMN	polymorphonuclear leukocytes
Poly(I:C)	Polyinosinic:polycytidylic acid
RANTES	regulated on activation, normal T cell expressed and secreted
SIRS	systemic inflammatory response syndrome
SPIHR	sustained peripheral immune hyperreactivity
STAT	signal transducer and activator of transcription
TLR	toll-like receptor
TNF	tumor necrosis factor

Learning Objectives

- Understand the basis of various intrauterine conditions that impact fetal development.
- Identify the mechanisms underlying the fetal inflammatory response.
- Recognize the significance of sustained immune hyperreactivity and its neuroinflammatory sequelae.
- Comprehend the mechanistic and clinical presentations of perinatal brain injury.
- Summarize the long-term cognitive outcomes associated with intrauterine inflammatory conditions.

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Highlights

- Chorioamnionitis encompasses diverse and widespread immune dysfunction throughout the placental–fetal brain axis.
- Sterile and infectious inflammation are equally important to the pathophysiology of brain injury commencing *in utero*.
- Perinatal brain injury reflects a multitude of initiating insults culminating in neural cell injury that leads to deficits in local, functional, and anatomical connectivity and pathological hallmarks at molecular, cellular, biochemical, structural, and ultrastructural levels.
- Systemic inflammation and neuroinflammation each contribute uniquely to the pathophysiology of perinatal brain injury and subsequent elaboration of neurodevelopmental impairment, including cerebral palsy and deficits of cognition and executive function

Section 1: An Adverse Intrauterine Environment

The intrauterine microenvironment plays a critical role in fetal development. This role relies tremendously on a precise homeostasis and is perhaps most conspicuous in cases where the intrauterine environment is disrupted or altered during pregnancy. While chorioamnionitis (CHORIO) and/or placental hypoxia-ischemia (HI) secondary to placental insufficiency are exceedingly common disruptions, issues with placental formation, placental stability and positioning, changes in amniotic fluid amount, maternal illness or trauma, toxin exposure, or genetic changes can each impact the maternal-placental-fetal brain axis and alter homeostasis during pregnancy, with putative consequences for neurodevelopment [1–4]. Due to the simultaneous presence of an immature fetal immune system, the developing central nervous system (CNS) is especially vulnerable to both the direct and indirect effects of many *in utero* conditions. Similarly, its protracted development throughout gestation and rapid period of development in the perinatal period *ex utero* increase the propensity for injury at all stages of pregnancy and in the postnatal period. As a result, the risk for the development of perinatal brain injury (PBI) is relatively prolonged and infants of all gestational ages are susceptible to modulations of the neural-immune system that can catalyze PBI (Fig. 9.1).

Chorioamnionitis (CHORIO)

For a large proportion of preterm infants with PBI, their injury begins *in utero* with CHORIO. Known to affect 40–80% of very preterm deliveries [5, 6], CHORIO is characterized by inflammation of the placenta and surrounding membranes. Although the precise definition of CHORIO is complex, controversial, and context dependent [6, 7], general definitions based on histological and clinical manifestations do exist. Clinical CHORIO is largely defined by maternal physiological factors, including fever, tachycardia, hyperleukocytosis, foul-smelling amniotic fluid or vaginal discharge, and uterine tenderness [8]. However, these maternal signs have variable correlation with clinical illness in the neonate, and may or may not be associated with histological CHORIO at all [9]. From a histological perspective, CHORIO is inflammation of the chorion and amnion. Diagnosis is based on neutrophil infiltration into these membranes and the decidua [7, 9–16]. Coupled with an elevation of intraamniotic cytokine levels, histological CHORIO may manifest in both the presence and absence of clinical signs in either the pregnant woman or fetus. Consequently, observations of consistent histological CHORIO and associated placental changes seen on placental pathology are up to three times as frequent as clinical CHORIO [6, 17–19]. Indeed, vascular, HI, and

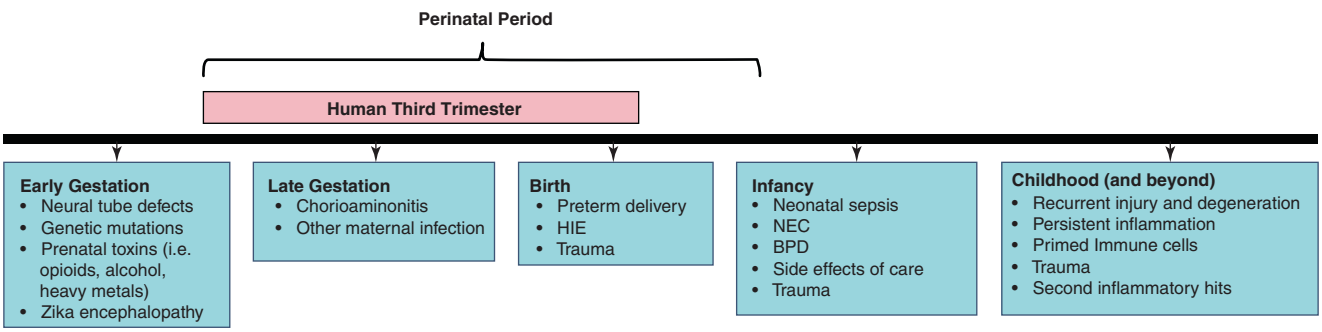

Fig. 9.1 Timeline of factors that contribute to brain injury. From early gestation through late childhood and beyond, numerous insults and conditions can develop that collectively contribute to neurological injury. These factors can be environmental, genetic, or biochemical in nature. A large number of these factors are introduced long before adulthood, but continue to have lasting neurodevelopmental effects, emphasizing the brain's vulnerability during the prenatal and perinatal periods

inflammatory changes are commonly observed in CHORIO placentas, even in the absence of demonstrable infection [6, 8, 18–20]. This highlights the importance of sterile inflammation and cellular changes in the placenta, independent of the microbial invasion and intrauterine infection that is classically thought to be associated with histological CHORIO and usually necessary for a clinical CHORIO diagnosis based on symptomology [21, 22]. Notably, there is tremendous overlap between inflammatory pathways throughout the body, whether or not infection is present. During sterile inflammation, placental cells under stress from HI or other microenvironmental signals can release Damage-Associated Molecular Patterns (DAMPS) that activate Toll-like receptors (TLRs) and induce cytokine production and recruitment of neutrophils in a similar manner to that which occurs with microbial invasion [7, 22].

Progression of Chorioamnionitis

Classically, CHORIO is induced through ascending microorganisms which catalyze an intraamniotic infection (Fig. 9.2). *Ureaplasma parvum and Ureaplasma urealyticum,* both genital mycoplasmas, are the most common bacteria isolated from the placentae and amniotic fluid of women with histological CHORIO. *Streptococcus hominis, Fusobacterium, Gardnerella vaginalis, and Escherichia coli* are also known to cause CHORIO [23–25]. Upon entering the body and establishing an ectopic infection, bacteria ascend to the subchorionic fibrin, or the interface between the chorion and the decidua layers of the placenta [7, 26, 27]. These bacteria release endotoxins and exotoxins which are recognized by TLRs on the surface of placental immune cells, including leukocytes, epithelial cells, and trophoblast cells [28, 29]. This surface recognition initiates intracellular pathways that activate transcription factors, such as nuclear factor kappa B (NF-kB), activator protein 1 (AP-1), and signal transducer and activator of transcription (STAT) that are central to inflammatory regulation. Their activation subsequently increases the production and release of pro-inflammatory cytokines, such as interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 1 alpha (IL-1a), interleukin 1 beta (IL-1b), and tumor necrosis factor alpha (TNF-a).

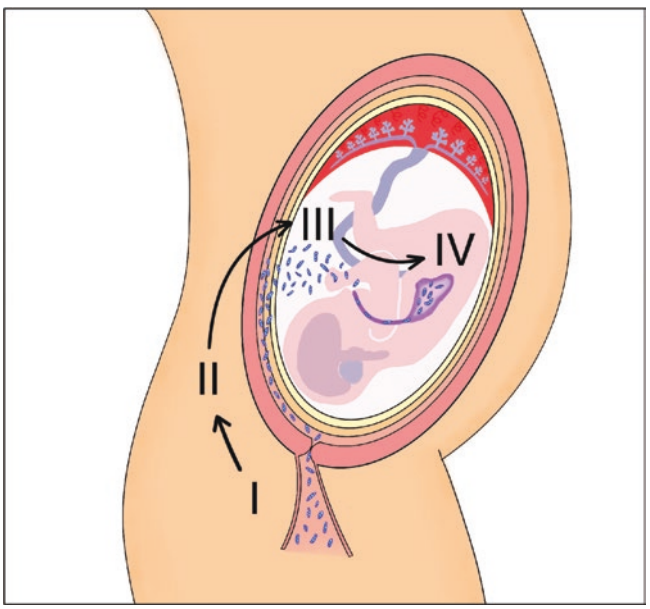

Fig. 9.2 The stages of ascending intrauterine infection (AIUI). In Stage I, bacteria/microorganisms enter the genital tract. In Stage II, bacteria infiltrate the choriodecidua. In Stage III, bacteria infiltrate the chorion and amnion, and enter the amniotic fluid. In Stage IV, bacteria enter the fetus via different ports of entry and may stimulate a fetal inflammatory response syndrome (FIRS). Modified from Fig. 7 in Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol. 2015;213(4 Suppl):S29–52

These cytokines, particularly IL-1b, propagate the expression of chemokines. Chemokines, a family of small molecular weight chemotactic cytokines [30–33], are classically defined by their ability to induce directional migration and activation of leukocytes to areas of inflammation in the body [30, 31, 34]. Chemokines are classified by their structural features. While CC chemokines contain two adjacent cysteines and predominantly attract monocytes and lymphocytes, CXC chemokines have one amino acid residue separating two conserved cysteines and attract neutrophils [30, 34]. C-X-C motif chemokine ligand 1 (CXCL1) is a chemoattractant for polymorphonuclear leukocytes (PMNs), such as neutrophils that is commonly expressed in response to pro-inflammatory cytokines [30, 35–38].

In pregnancy and fetal development, the physiologic roles for chemokines are well described [39–41]. Neutrophils express IL-8, C-X-C motif chemokine ligand 6 (CXCL6), and C-X-C motif chemokine receptor 2 (CXCR2), the cell surface receptor for CXCL1. Thus, as subchorionic infection upregulates chemokine production, these chemokines recruit neutrophils, monocytes, and macrophages from the maternal circulation to infiltrate the choriodecidua, propagating intrauterine inflammation [7, 42, 43]. Subsequently, cytokines, immune cells, and microorganisms themselves migrate deeper into the uterus, traversing the decidua, chorion, and amnion before finally invading the amniotic fluid (Fig. 9.3) [7, 26]. Once the bacteria disperse in the amniotic fluid, they make widespread contact with the chorion, amnion, fetal skin, and umbilical cord, activating TLRs on epithelial cells.

In most cases of CHORIO, neutrophils are the defining cellular signature in the decidua. However, CHORIO is also defined by a diverse expression of pro-inflammatory cytokines, each with their own role in recruiting immune cells and propagating inflammatory signal transduction that dis-

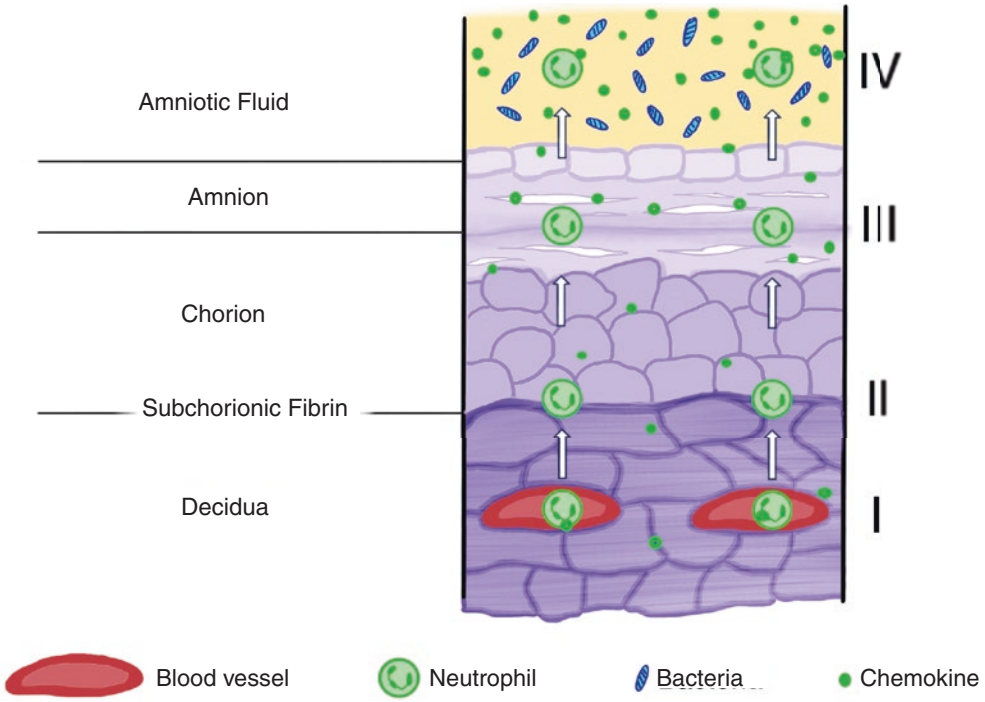

Fig. 9.3 Migration of neutrophils from the decidua to the amniotic Fluid. In phase I, maternal neutrophils reside in the decidual blood vessels. In phase II, neutrophils migrate into the subchorionic fibrin, following a chemokine gradient. At this point, mild histologic CHORIO, defined by acute subchorionitis/chorionitis, is established. In phase III, neutrophils reach the connective tissue between the chorion and amnion. At this point, moderate histologic CHORIO is established. In phase IV, neutrophils reach the amniotic fluid, where the highest concentration of chemokines is. Modified from Fig. 9.3 in Kim CJ, Romero R, Chaemsaithong P, Chaiyasit N, Yoon BH, Kim YM. Acute chorioamnionitis and funisitis: definition, pathologic features, and clinical significance. Am J Obstet Gynecol. 2015;213(4 Suppl):S29–52

rupts uterine homeostasis. Importantly, dysregulated cytokine production due to infection can have a tremendous impact on the fetus and may potentially harm the developing fetal brain [5, 16, 44–53]. Studies have shown that intraamniotic, cord blood, or maternal blood levels of IL-6, a pluripotent cytokine, may serve as a biomarker for the severity of CHORIO [54–56]. However, recent studies have focused on cytokine and chemokine diversity and networks of these proteins as opposed to any one cytokine in isolation. For example, human clinical studies have found elevated levels of IL-6, IL-8, matrix metalloproteinase 8 (MMP-8), and CXCL1 in the amniotic fluid of women with CHORIO [57]. In particular, CXCL1 levels are known to positively correlate with the severity of pathologic placental inflammation and are a biomarker for microbial invasion of the amniotic cavity [58, 59]. Indeed, preclinical studies of CHORIO have confirmed unique roles for many pro-inflammatory proteins, each with their own compartment-specific expression pattern, including IL-1b, IL-6, TNF-a, CXCL1, matrix metalloproteinase 10 (MMP-10), C-C motif chemokine ligand 2 (CCL2) also known as monocyte chemoattractant protein 1 (MCP-1), and cyclooxygenase-2 (Cox-2) [35–37, 60]. The importance of cytokines in the context of CHORIO cannot be overstated, especially in the context of risk for subsequent effects on neurodevelopment and PBI. For example, in the absence of other risk factors, the cytokine milieu of CHORIO can facilitate a hypercoagulable state or cause direct thromboembolism to the fetal brain [14, 61]. Cellular and molecular placental abnormalities associated with CHORIO are also an independent risk factor in the pathogenesis of perinatal stroke [62–66]. These data stress the cumulative importance of inflammation itself in homeostatic regulation of the placental microenvironment and subsequent risk for the pregnancy as a whole.

Chorioamnionitis and Preterm Labor

As described previously, cytokines, such as IL-6 and chemokines, such as CXCL1, serve as chemoattractants for monocytes and neutrophils and reflect a complex immune microenvironment in the context of CHORIO. By virtue of their chemoattractant roles, immune cells, including neutrophils migrate from the decidual vessels, through the chorion and amnion, and into the amniotic fluid as chemokine concentrations rise. Elevated cytokine concentrations within the amniotic fluid then facilitate immune activation and transfer to the fetus. This pathological inflammation also serves as a mechanism to induce premature rupture of membranes and preterm delivery. During intrauterine infection, upregulated

cytokines stimulate the production of prostaglandins and induce uterine contractions [28]. They may also activate matrix metalloproteinases that catalyze cervical ripening and membrane rupture. Notably, prostaglandins can be inactivated by prostaglandin dehydrogenase, but infection of the chorion inhibits the release of this key enzyme, allowing prostaglandins to travel to the myometrium [28]. As such, CHORIO is the most common abnormality found in preterm deliveries [67–69].

Chorioamnionitis and Term Birth

While CHORIO is most commonly associated with preterm birth, it is also reported to occur in 3–10% of term pregnancies [70, 71]. In this context, CHORIO is strongly associated with the development of perinatal arterial ischemic stroke and supports a severe inflammatory process yielding predisposition to ongoing perinatal HI [72]. Interestingly, in term infants with HI encephalopathy (HIE), the presence of CHORIO predicts decreased responsiveness to hypothermia treatment [28, 51, 73–76]. In this respect, CHORIO has also been associated with increased risk of CP in term infants [77]. Cumulatively, these data indicate an intricate overlap between intrauterine inflammation and subsequent injury independent of gestational age.

Placental Insufficiency and Intrauterine Growth Restriction

Intrauterine growth restriction (IUGR), defined as a fetal weight below the tenth percentile for gestational age [78], is another common intrauterine issue that affects approximately 20 million infants each year [79]. IUGR is usually caused by placental insufficiency (i.e., HI), a condition in which placental efficiency decreases and fetal oxygen and nutrient delivery is impaired [80]. There are various etiologies of placental insufficiency leading to IUGR, including both fetal and maternal factors. These include chromosomal abnormalities, congenital fetal abnormalities, multiple gestations, infection, poor maternal nutrition, maternal vascular disease, and maternal substance abuse [81, 82]. Placental insufficiency also leads to chronic hypoxia [83]. The fetus adapts by redistributing cardiac output to vital organs, such as the brain, thereby compromising growth [82]. Despite these adaptations, studies show that chronic hypoxia and inflammation still have an impact on brain structure and function. Reduced oxygen availability leads to inflammation, excitotoxicity, and oxidative stress, culminating in mitochondrial injury, neural injury, and cell death [80, 84]. With respect to neuroinflammation in IUGR, the literature is limited and studies examining neonates are often influenced by other factors, such as prematurity and infection [84]. Examination of umbilical cord blood in growth restricted neonates shows elevations in interferon gamma (IFN-γ) [85], a robust pro-inflammatory cytokine. Similarly, animal models of chronic hypoxia demonstrate elevations of TNF-a and IL-1b that correlate with the severity of brain injury [86].

While its etiology is multifactorial, placental insufficiency, like CHORIO, often results in long-term neural connectivity deficits, structural brain alterations, and impaired brain function [83, 87, 88]. Studies in humans have shown that infants with IUGR have decreased total brain volume, reduced gray and white matter volumes, decreased myelination, smaller hippocampal and cerebellar volume, and reduced connectivity [88]. Functional deficits are seen alongside these changes, and are further discussed with respect to long-term outcomes associated with IUGR. Animal studies examining cellular changes associated with growth restriction confirm neural cell death alongside abnormal maturation of oligodendrocytes and disrupted myelination, which in turn interfere with proper white matter connectivity [88]. Cumulatively, these changes impact neurodevelopment. The timing of growth restriction, severity of growth restriction, and degree of prematurity at birth impact the severity of neurodevelopmental outcomes, including motor impairment, sensory impairment, cognitive disability, and CP [88–90].

Section 2: Fetal Inflammatory Response Syndrome and Systemic Inflammatory Response Syndrome

While placental insufficiency causes sterile inflammation through HI, indirectly damaging tissues through the depletion of blood, oxygen, and glucose, CHORIO directly causes placental inflammation and cumulative dysfunction of inflammatory signal transduction traversing the maternal–placental axis. In severe cases, CHORIO can induce a fetal inflammatory response syndrome (FIRS), which often has a significant impact on the developing brain. FIRS occurs when inflammation spreads from the amniotic cavity to the fetus itself and is defined by systemic activation of the fetal innate immune system and a profound systemic cytokine response [91, 92]. Historically, FIRS has been described as an elevated IL-6 concentration in the umbilical cord plasma and/or the presence of funisitis [12], although the role of other inflammatory mediators, such as CXCL1 and CCL2, is increasingly appreciated.

Progression of FIRS

Like CHORIO, the pathophysiology and mechanisms of FIRS are exceedingly complex. However, the initiation of

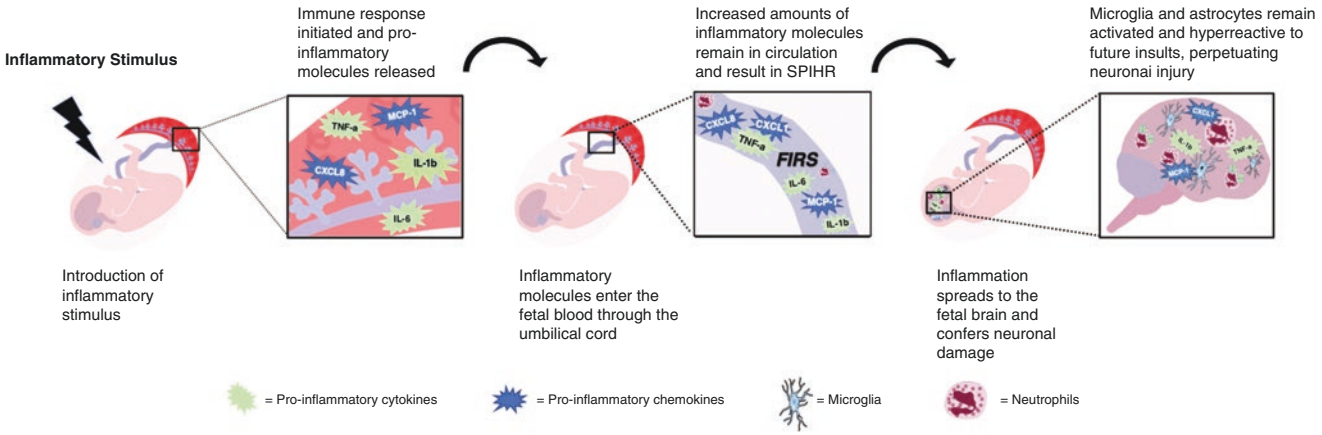

Fig. 9.4 Propagation of Inflammatory Signal Transduction Through the Placental-Fetal-Brain Axis. An initial insult causes an inflammatory response that leads to an increase of pro-inflammatory molecules in the placental and subsequently in the fetal blood, resulting in FIRS. Inflammation, through a robust cellular and molecular communication network in the body, confers neural cell injury. Peripheral immune cell, microglia and astrocytes become activated and may become responsive (SPIHR) to subsequent insult or immune challenges further perpetuating CNS injury

FIRS is an important catalyst to injury to the developing CNS (Fig. 9.4). There are two primary mechanisms thought to play prominent roles. First, bacterial microorganisms, neutrophils, and other immune cells and cytokines in the maternal circulation can directly enter the fetal circulation via the umbilical cord [93–95]. In response to the invading microorganisms circulating throughout the fetus, additional inflammatory proteins and activated immune cells are recruited from the maternal circulation, through the placenta, and into the fetal circulation, inciting a FIRS [96]. The second, less direct mechanism is more frequently described in the literature [7, 97]. In this scenario, FIRS is propagated as the fetus breathes and swallows amniotic fluid. Fetal epithelial organs, such as the skin, lungs, and gut, are then exposed to bacteria and cytokines in the amniotic fluid. As described above, TLRs on these exposed cells are activated, causing greater cytokine production and immune cell recruitment. These proteins and cells then traverse the fetal circulation [16, 93, 96, 98].

Many clinical studies validate the occurrence and importance of FIRS secondary to histologic and/or clinical CHORIO. Neonates born to mothers with cervical-vaginal infection and/or CHORIO have been shown to have elevated c-reactive protein (CRP), myeloperoxidase (MPO), CCL2 (MCP-1), matrix metalloproteinase 9 (MMP-9), IL-1b, IL-6, TNF-a, and C-C motif chemokine ligand 5 (CCL5) (also known as Regulated Upon Activation Normal T Cell Expressed and Secreted, or RANTES) in their blood [99, 100], consistent with monocyte, macrophage, neutrophil, and T-cell recruitment. Furthermore, concentrations of IL-6 and TNF-a in the amniotic fluid have been shown to be predictive of FIRS [101], and high amniotic fluid CXCL1 concentrations correlate with maternal and newborn white blood cell counts [102]. Preclinically, rat models with an intact maternal–placental–fetal axis have similarly demonstrated a systemic fetal inflammatory response in settings of CHORIO, as evidenced by elevated IL-1b, TNF-alpha, IL-6, interleukin 10 (IL-10), and CXCL1 in the serum of developing pups [35–38].

Funisitis Develops Concomitantly with FIRS

Concomitant with FIRS is funisitis. Widely accepted as the histological counterpart to FIRS, funisitis is defined as inflammation of the umbilical cord due to infiltration of fetal neutrophils [26, 57, 103]. While CHORIO can be conceptualized as a maternal inflammatory response, funisitis is a fetal inflammatory response, with infiltrating neutrophils originating from the fetal side [7, 101, 104]. Funisitis has a well-documented progression, wherein inflammation begins in the sole umbilical vein, before spreading to the umbilical arteries and finally the Wharton's jelly [7, 26]. Notably, a stepwise increase in CHORIO, FIRS, and suspected early-onset neonatal sepsis has been observed clinically as funisitis progresses [26]. In this respect, funisitis may be viewed as the last stage of ascending intrauterine inflammation (AIUI), and a potential, emerging biomarker for severe CHORIO and FIRS [26]. Funisitis is also associated with neurodevelopmental disorders, including cerebral palsy.

Systemic Inflammatory Response Syndrome

The transition from FIRS to a systemic inflammatory response syndrome (SIRS) occurs *ex utero*. Notably, how-

ever, both FIRS and SIRS have similar defining characteristics, including cytokine elevation, immune cell activation which alters neural-immune cross-talk, and the potential for neural cell injury. In addition, both FIRS and SIRS can lead to sustained immune hyperreactivity within the neonate, sensitizing its immune system to future CNS insult and injury. The elaboration of SIRS or early-onset sepsis is often defined clinically by observable symptoms within the first 72 h of life. Diagnostic criteria include a core temperature greater than 38.5° C or less than 36° C, tachycardia or bradycardia, high mean respiratory rate, and an elevated or depressed leukocyte count [105]. By contrast, late-onset neonatal sepsis, defined by clinical symptoms appearing more than 3–7 days after birth, is usually attributed to postnatal acquisition of infection as opposed to CHORIO or in utero infection/inflammation [106]. Indeed, rates of SIRS or early-onset neonatal sepsis increase substantially in low birthweight infants when maternal CHORIO is present [106]. SIRS is particularly common in preterm infants, and may act as a second hit that severely impairs postnatal CNS development, resulting in lifelong neurodevelopmental disabilities [107–109]. Similar to CHORIO and FIRS, SIRS is highly associated with PBI and susceptibility to CP [107, 110, 111].

Section 3: Sustained Peripheral Immune Hyperreactivity

In utero inflammation secondary to CHORIO or placental insufficiency can exert long-term effects on immune function that manifest as sustained inflammation within the fetus and durable changes to immune cells during the perinatal period. Inflammation is essential for recruiting immune cells and growth factors to an area of injury for recovery, repair, and regeneration [43, 52, 53, 112]. However, when inflammation becomes prolonged, severe, or unchecked by anti-inflammatory factors, it can exacerbate injury, enhancing susceptibility to CNS and other organ damage. With respect to the brain, when neuroinflammation becomes prolonged or severe, insult is propagated through an additional influx of cytokines, chemokines, and other inflammatory mediators released from glial cells [43]. The same is true in the periphery, where in addition to these processes, lymphocytes can become hyperresponsive and feed forward sustained peripheral immune hyperreactivity (SPIHR) (Fig. 9.5) [113–115]. Defined by an exaggerated immune response to secondary inflammatory insults and a degree of immune cell memory, SPIHR is an additional mechanism by which vulnerability to brain injury can be conferred. This peripheral immune hyperreactivity is particularly pronounced in neonates following infection or injury, such as former preterm infants with cerebral palsy or children with Trisomy 21 [113, 116, 117].

Evidence for SPIHR

SPIHR, characterized by sustained immune cell activation and priming, results in positive feedback loops that elevate cytokine secretion and recruit additional immune cells. Pro-inflammatory cytokines can be assayed at various time points after neonatal infection to examine the trajectory of the immune response, characterize SPIHR, and gain insight into the relative activity of individual chemokines over time. In elegant clinical studies by Nguyen et al., secreted cytokine profiles were compared in neonatal neutrophils versus adult neutrophils following exposure to lipopolysaccharide (LPS), a bacterial endotoxin [118]. This investigation revealed that neonatal neutrophils secreted higher levels of IL-8 than adult neutrophils, both 0 h and 24 h after LPS stimulation [118]. In a separate set of studies, Lin et al. used LPS to stimulate peripheral blood mononuclear cells (PBMCs) isolated from former preterm infants with and without CP at school age. PBMCs collected from former preterm children with CP were found to hyper-secrete TNF-alpha in response to LPS challenge when compared to PBMCs from children without CP [113].

These results have been replicated in an established preclinical rat models of CHORIO and perinatal opioid exposure [114, 115]. Specifically, Newville et al. compared the secretory cytokine profiles of PBMCs sampled from rats exposed to methadone versus saline during the perinatal period. Upon secondary LPS stimulation, PBMCs isolated from rats exposed to methadone *in utero* exhibited a significantly greater increase in secreted cytokines, such as TNF-alpha, CXCL1, IL-6, and IL-10 compared to controls [114]. These results point to sustained immune hyperreactivity and suggest a lasting impact of inflammation from in utero exposures. Similarly, Yellowhair et al. used a secondary hit of LPS to stimulate PBMCs from rats exposed to prenatal CHORIO and sham controls at both postnatal (P) day 7 (term equivalent) and P21 (toddler equivalent) [115]. Upon LPS stimulation at P7, CHORIO PBMCs were found to secrete significantly higher levels of TNF-a and CXCL1 compared to sham PBMCs. LPS stimulation at P21 demonstrated similar results, with CHORIO PBMCs secreting significantly higher levels of TNF-a and IL-6 compared to sham controls. In addition, P21 non-stimulated CHORIO PBMCs had significantly higher TNF-α secretion compared to sham PBMCs at 24 hours, indicating changes in baseline inflammatory reactivity and secondary immune stimulation [115]. Together, these data suggest that in settings of persistent immune hyperreactivity, the introduction of a secondary inflammatory stimulus triggers an exaggerated peripheral immune response, substantiating the presence of SPIHR in neonates previously exposed to intrauterine inflammation (Fig. 9.5).

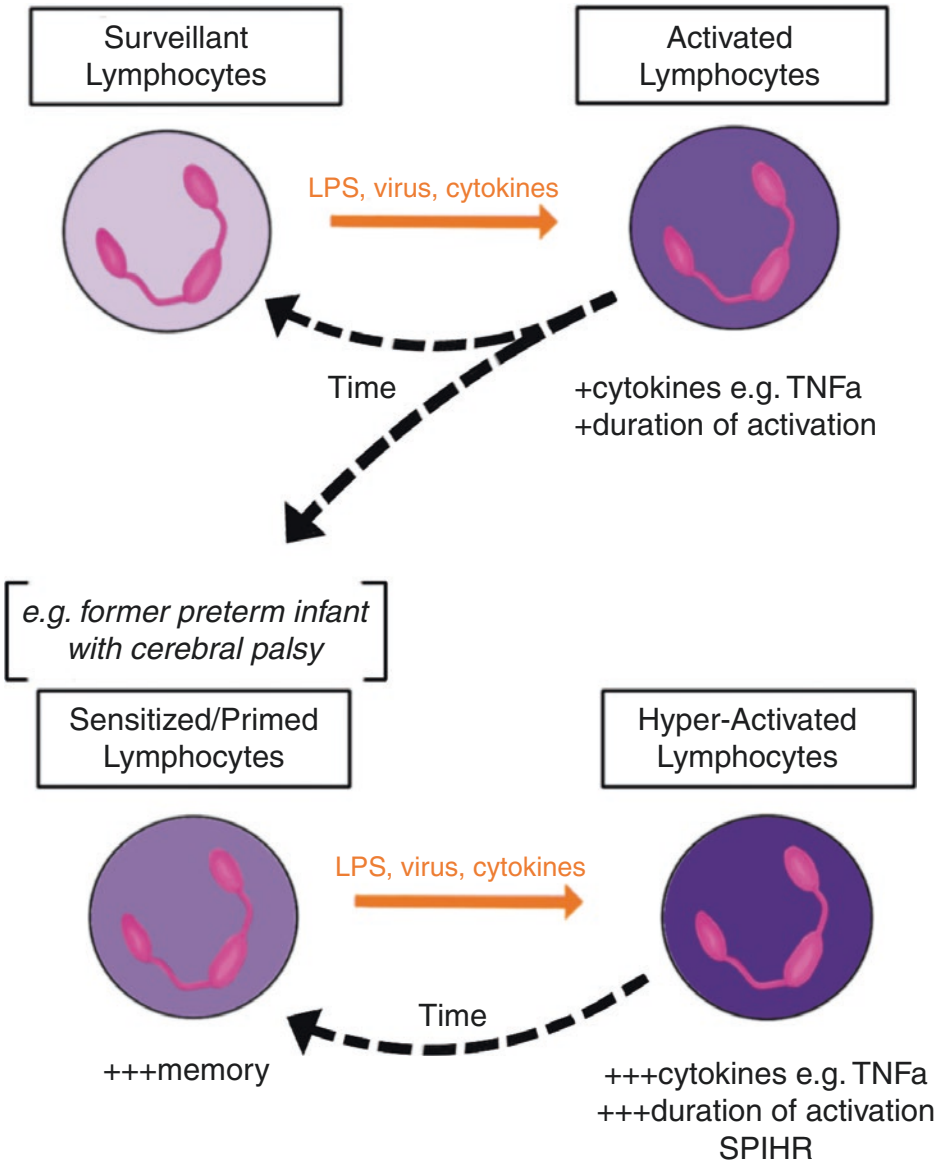

Fig. 9.5 Sustained Peripheral Immune HyperReactivity (SPIHR). Sustained activation of peripheral lymphocytes. Inflammatory stimuli (LPS, virus, cytokines) trigger surveillant or resting lymphocytes to become activated lymphocytes. Activated cells secrete cytokines. After being activated for a certain amount of time, lymphocytes may either return to their surveillant state, or remain sensitized/primed. Lymphocytes in a former preterm infant with cerebral palsy, for example, would have sensitized/primed immune cells. These primed cells retain memory of the original inflammatory stimulus, and a secondary inflammatory stimulus may trigger these cells to enter a hyperactivated state. In a hyperactivated state, cells secrete even more cytokines than in their activated state. This hyperreactive response to secondary stimuli is characteristic of SPIHR. After a longer period of time, these cells may return to their primed state

Peripheral Inflammation Infiltrates the CNS

Peripheral inflammation, though necessary for acute physiological response to injury, can be especially damaging when it spreads to the CNS. However, the bridge between systemic inflammation and neuroinflammation, especially in infants, is multifactorial and exceedingly complex. The presence of an intact blood–brain barrier (BBB) typically prevents direct leukocyte trafficking into the brain. However, the integrity of the BBB is often compromised in tandem with excess production of pro-inflammatory molecules and immune cell activation. Additionally, the BBB itself has a precise developmental trajectory involving astroglia, pericytes, the extracellular matrix, and tight junctional proteins. Recent studies have pointed to extracellular vesicles as potential propagators of neuroinflammation, as these vesicles can travel peripherally and cross the BBB [119, 120]. Specifically, extracellular vesicles known as exosomes can carry cargo, including lipids, cytokines, mRNA, microRNA, and growth factors [120–122]. They interact with other cells through ligand–receptor binding or membrane fusion, which allows for content transfer between other cells throughout the body. In the context of systemic inflammation, exosomes are speculated to act as the bridge between the periphery and the

CNS, transporting cytokines, viruses, or other inflammatory molecules across the BBB [122, 123]. In the CNS, microglia and astrocytes also secrete exosomes that transport miRNA, cytokines, and chemokines. Studies in humans have shown that glial cells can release exosomes containing inflammatory molecules, such as IL-1β, and astrocyte-derived exosomes can transport pathogenic proteins and aberrantly expressed miRNAs to neurons. These processes initiate or propagate neuroinflammation [121, 124]. Similarly, human microglia can secrete pro-inflammatory cytokines, such as IL-1β, when stimulated with extracellular vesicles isolated from the serum of patients with inflammatory conditions [125]. In mice, inhibition of exosome release from cells of the CNS attenuates systemic peripheral immune response to neuroinflammation, while inhibiting leukocyte infiltration [119, 126].

Given that exosomes appear to serve as important mediators of the interplay between peripheral and CNS inflammation, they may also play a significant role in the pathophysiology of CHORIO and inflammatory signal transduction across the maternal–placental–fetal axis. Exosomes traverse the maternal–fetal interface in order to establish a relatively immune-privileged milieu for fetal development [120]. In addition, amniotic fluid exosomes provide information specific to normal and abnormal parturition and inflammatory intrauterine microenvironments [127, 128].

CNS Immune Response – Neuroinflammation

Once cytokines and inflammatory cells enter the CNS, astrocytes and microglia play significant roles in staging inflammatory responses. Astrocytes are specialized glial cells which are critical for essential functions of the CNS and maintenance of the BBB. In the case of direct injury to the CNS, via HI or traumatic brain injury, for example, these cells respond through a complex process termed astrogliosis. During astrogliosis, astrocytes alter their gene expression, cellular structure, and function in response to CNS damage [129]. In infants with HI brain injury, up to 40% show signs of astrogliosis within white matter [130]. Furthermore, when peripheral inflammation infiltrates the CNS, astrocytes respond to such signals by propagating the release of more pro-inflammatory cytokines, triggering a larger inflammatory response within the brain. Indeed, mouse models of peripheral infection have shown rapid astrocyte activation in response to peripheral immune activation elicited by either LPS or Polyinosinic:polycytidylic acid (poly(I:C)) [131]. Moreover, in a preclinical rat CHORIO model, Jantzie et al. found increased astroglia in CHORIO animals compared to controls [38, 132].

By contrast, microglia are the major inflammatory mediators of the brain and shift into various functional states based on the conditions of their microenvironments (Fig. 9.6). Their ability to exhibit phagocytic behavior, bind and release cytokines and chemokines, and proliferate rapidly make them critical for instigating CNS inflammation, prompting neurogenesis, and maintaining homeostasis [133]. Microglia can polarize into phenotypes from their resting state. In response to LPS or IFN-γ, microglia can morph into an "M1"-like phenotype which propagates the expression of pro-inflammatory cytokines [134–138]. On the other hand, in response to interleukin 4 (IL-4) and interleukin 13 (IL-13), for example, microglia can transform into an "M2"-like state, which is believed to be important for the resolution of inflammation and tissue repair [134–138]. Similar to peripheral macrophages, the polarization of microglia is likely a continuum and represents complex phenotyping involving many intermediate stages.

Sustained CNS Inflammation

Unlike neutrophils, which typically survive in humans for no longer than about 5 days, microglia turn over at a much slower rate, allowing them to remain activated for months or even years after initial injury or infection, in a manner that parallels SPIHR. Their prolonged activation can lead to the persistent production of reactive oxygen species that contribute to oxidative stress and pro-inflammatory cytokine production that perpetuates neural cell injury [139]. Various studies also demonstrate that microglia become primed by ongoing neuropathology in the brain, which increases their response toward subsequent inflammatory stimuli, including systemic peripheral inflammation [140, 141]. Secondary systemic inflammation can switch these primed microglia from a relatively idle to hyperactive state through the upregulated secretion of pro-inflammatory cytokines (Fig. 9.6) [142]. Studies of microglia in preclinical models of CHORIO have shown that inflammatory exposure during fetal life significantly increases microgliosis, thereby inducing microstructural and diffusion abnormalities in white and gray matter [37, 38]. Overall, these findings strongly point toward sustained, hyper-reactive immune activity in the CNS following injury or infection and emphasize the importance of protein and cellular mediators at all stages of inflammation (Table 9.1).

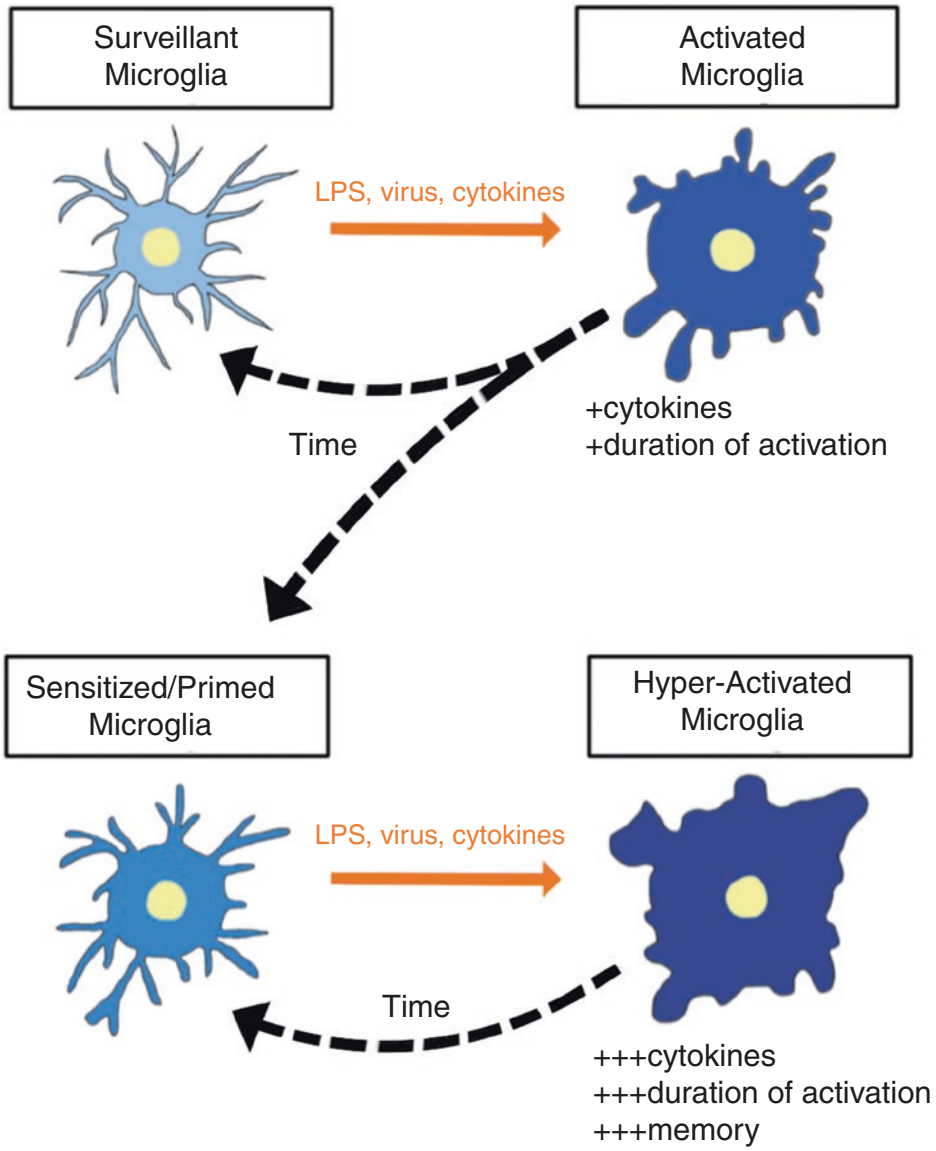

Fig. 9.6 Sustained activation of microglia in the CNS. Inflammatory stimuli (LPS, virus, cytokines) trigger surveillant or resting microglia to become activated. Activated cells secrete cytokines among other proinflammatory proteins. After being activated for a certain amount of time, activated microglia may either return to their surveillant state, or remain sensitized/primed. A secondary inflammatory stimulus may trigger primed cells to enter a hyper-activated state. In a hyperactivated state, microglia secrete even more cytokines than in their activated state, and retain memory of the stimulus

Table 9.1 Key molecular and cellular mediators in chorioamnionitis

Molecular Inflammation	Cellular Inflammation
Pro-inflammatory cytokines (eg IL-6, TNF-a)	Microglia
Anti-inflammatory cytokines (eg IL-10)	Monocytes
Pro-inflammatory chemokines (eg. CXCL1, CCL2)	Macrophages
	T-cells
	Neutrophils

Section 4: Neonatal Brain Injury

PBI is a major contributor to long-term disability in children across the globe [143]. More than half of those infants surviving PBI have chronic neurological conditions, such as CP and cognitive delay [111, 144–147]. For a large proportion of infants with PBI, CNS injury begins *in utero* with inflammation and/or HI. CHORIO not only contributes to preterm CNS injury but is also a common, independent risk factor for term infant brain injury, including perinatal stroke [5, 61, 62, 65, 66, 148, 149]. Neurological deficits are frequently cumulative in children with PBI. That is, patients with cognitive and behavioral problems often have CP, impaired learning, epilepsy, and poor physical health as well, contributing to chronic disease in adulthood [150–153]. Recently, it has been recognized that current prenatal care and prevention efforts are ineffective in reducing the burden associated with neonatal mortality and morbidity, highlighting the necessity of identifying underlying pathophysiological mechanisms and testing novel neural repair strategies [154, 155].

Infection and HI catalyze PBI by creating a toxic microenvironment that limits oxygen exchange and propagates inflammation during critical periods of neurodevelopment [13, 14, 28, 73, 74, 156–158]. Typically, infants with PBI

Table 9.2 Key determinants and heterogeneity of brain injury in preterm infants

Preterm Infants					
Connectivity loss	Anatomical/structural	Functional			
Neural network fragmentation	Biochemical	Molecular	Microstructural	Ultrastructural	
Sequelae	Cerebral palsy	Cognitive deficits	Executive function abnormalities	Chronic pain	SPIHR

present with injury to major white and gray matter structures, leading to reduced connectivity of developing networks. Subsequently, diverse functional deficits ensue with impairment in multiple motor, cognitive and emotional realms, including educational underachievement in childhood (Table 9.2) [5, 159–163]. Risk of brain injury is elevated in infants with persistent and/or recurrent elevations of pro-inflammatory proteins [164]. Specifically, the increasing breadth of early neonatal inflammation, indexed by the number of elevated proteins or functional protein classes, is associated with increased structural and functional brain injury [67, 68, 164–168]. Infants with CHORIO have elevated neutrophil and monocyte counts compared to infants without intraamniotic infection [100], emphasizing compartment-specific modulation of cytokines and immune cell diversity in CHORIO. Typically, neutrophil influx is a secondary response after brain injury, which further exacerbates endogenous inflammation mediated by microglia [169, 170]. Microglia have also been strongly implicated in the pathophysiology of white matter injury and CP.

Section 5: Long-Term Outcomes

While PBI reflects a diversity of initiating insults that impact the intrauterine environment and the maternal–placental–fetal axis, common hallmarks of PBI do exist, including injury to developing oligodendrocytes, axons, migrating GABAergic neurons, and subplate neurons [111]. Similar to this diverse cell-type specificity, there is a heterogeneity in brain region involvement, with the thalamus, white matter, cortex, basal ganglia, cerebellum, and spinal cord each being affected. Cumulatively, this yields inherent issues in neural networks and diminished structural, functional, and local connectivity. Decreased integration, transmission capacity, global efficiency, and specialization in the developing brain manifests as impaired cognitive, executive, motor, and neuropsychological outcomes across the lifespan [171–173].

Cerebral Palsy

Cerebral palsy (CP) is the most common cause of childhood-onset, lifelong physical disability. Its clinical manifestations vary widely in the type of motor impairment, the degree of functional ability and limitation, and the area of the body affected [147, 174, 175]. The etiology of CP includes CHORIO and preterm birth, maternal and neonatal infections, IUGR, neonatal encephalopathy related to hypoxic ischemia, infant traumatic brain injury, and genetic causes. As described previously, the sequelae and downstream effects of CHORIO, including FIRS, SIRS, and funisitis, are associated with CP. Preterm birth is the most significant risk factor for CP, and the risk is inversely correlated with gestational age [176, 177]. Despite improved perinatal care, it is estimated that 5–10% of preterm infants develop CP and 50% of preterm infants suffer from cognitive and behavioral disorders [178]. Most individuals with CP experience lifelong neurological comorbidities, including movement disorders, epilepsy, chronic pain, and difficulties with speech, cognition, and behavior [179, 180]. In addition, adult CP patients have a significantly higher risk of depression and anxiety [181]. Clearly, the *in utero* inflammation that these individuals experience during the preterm period can persist as SPIHR for a long period of time, increasing the propensity for chronic neurological disorders throughout the lifespan [113].

Posthemorrhagic Hydrocephalus Secondary to Intraventricular Hemorrhage

Like CP, CHORIO heightens preterm infants' risk of developing severe intraventricular hemorrhage (IVH) [182–185]. Over time, generally weeks, this IVH can either self-resolve or transform into symptomatic posthemorrhagic hydrocephalus (PHHP) requiring surgical intervention. Globally, it has long been known that IVH occurs more frequently in very preterm neonates prenatally exposed to chorioamnionitis [182–189]. Similar to CHORIO, the development of neonatal sepsis correlates with advancement to PHHP, implicating systemic inflammation as a key driver in this progression [190]. Indeed, it was discovered that neonatal sepsis trends along the same curve as infants with IVH who progress from ventriculomegaly to symptomatic PHHP requiring surgical intervention [190]. Notably, prenatal models suggest that blood-borne inflammation can cause hydrocephalus [191], and adult preclinical models demonstrate that systemic inflammation increases choroid plexus secretion of CSF [192]. More recently, it has also been shown that inflammation reduces ependymal motile cilia propulsion of CSF [191, 193, 194] and CSF reabsorption by the glymphatic system [195]. Together, these findings implicate systemic inflammation as the key trigger that switches IVH from a course of spontaneous recovery to progression to symptomatic PHHP.

Cognitive Impairment/Executive Dysfunction

In examining outcomes associated with PBI, it is clear that neurocognitive deficits, especially those associated with executive function, have a profound impact on individual functioning, families, and society as a whole. Studies examining long-term outcomes of cognitive impairment and behavioral dysfunction demonstrate that degree of prematurity, growth restriction with placental insufficiency, and perinatal infection act synergistically. Indeed, almost half of infants born at less than 28 weeks gestational age demonstrate significant cognitive delay and behavioral problems [196, 197]. These deficits are more pronounced in premature infants who are also small for gestational age [196]. IUGR infants across all gestational ages demonstrate impairment in cognitive and behavioral domains. However, the severity of impairment has been shown to be greater in infants with IUGR born at less than 35 weeks gestational age [198]. Additional studies also point to cognitive, motor, social, attentional, and memory issues in school-age children with IUGR [199, 200]. With respect to direct inflammatory insults, perinatal infection augments risk for cognitive impairment in extreme prematurity and many studies have demonstrated that children exposed to histologic and/or clinical CHORIO have lower cognitive, language, and motor scores within the first years of life compared to controls [93, 201, 202]. Notably, severe FIRS, evidenced by necrotizing funisitis and chorionic plate vasculitis with thrombosis, is also associated with severe neurodevelopmental impairment [183]. In a systematic review of 18 studies, the presence of an ascending intrauterine infection with funisitis was found to be the most consistent placental feature accompanying poor neurodevelopmental outcomes in children born preterm and term at 2 years of age [203]. These outcomes included moderate to severe psychomotor disability, speech abnormalities, and hearing loss [203]. In addition, population cohort studies have shown that the risk for abnormal neurologic outcome at 24 months is highest for extremely preterm infants exposed to both histologic CHORIO and placental insufficiency [204]. Considering this evidence together, it is clear that early recognition of adverse intrauterine events is an important first step in improving care for infants in the NICU.

Chapter Summary

The establishment and maintenance of pregnancy relies on a delicate homeostasis. Of critical importance is placental homeostasis and the balance between pro- and anti-inflammatory factors within the maternal–placental–fetal axis. While the successful delivery of an infant requires multiple signaling pathways, cells, and inflammatory molecules, the balance, timing and location of these factors are key. When homeostatic regulation of inflammatory cells and signaling molecules is perturbed, pathologies common in pregnancy emerge. CHORIO is defined by robust placental inflammation and an upregulation of diverse inflammatory networks. This inflammation usually does not self-resolve, and subsequent dysfunction in the maternal–placental–fetal axis can have consequences for the success of the pregnancy, fetal brain development, and fetal immune maturation. Indeed, CHORIO is a common, independent risk factor for brain injury in both term and preterm infants and outcomes are worse in pregnancies with CHORIO and HI/placental insufficiency combined [148, 149]. To this end, minimizing PBI requires identification of critical pathways underlying the developmental program shared by both the placenta and developing brain. It also relies on the continued development of precision placental, liquid, neuroimaging, and biobehavioral precision biomarkers. Not only will these biomarkers inform diagnosis and the design of future clinical trials aimed at improving long-term outcomes, but they could also estimate the extent of immune system abnormalities and CNS injury. In this context, a rigorous, complimentary, and coordinated approach with precision medicine is needed to transform the care of children with brain injury commencing *in utero*.

Multiple Choice Questions

1. What is the histological counterpart to FIRS?
   A. SIRS
   B. **Funisitis**
   C. Placental Insufficiency
   D. Chorioamnionitis
2. What extracellular vesicles are speculated to be responsible for transferring peripheral inflammation to the CNS?
   A. **Exosomes**
   B. Leukocytes
   C. Cytokines
   D. Microvesicles
3. Which cells are most commonly associated with SPIHR?
   A. Endothelial Cells
   B. Exosomes
   C. **Lymphocytes**
   D. Astrocytes
4. Which cells are the defining feature of histologic chorioamnionitis?
   A. **Neutrophils**
   B. Microglia
   C. Astrocytes
   D. T-cells
5. Which neural cells are vulnerable to brain injury secondary to chorioamnionitis?
   A. Oligodendrocytes

B. Neurons
C. Microglia
D. **All of the above**

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Stress as a Determinant of Neurodevelopmental Outcomes

10

Bona Kim and Stephen G. Matthews

Learning Objectives

1. Learn about the long-lasting neurodevelopmental phenotypes observed in children following prenatal maternal stress.
2. Understand the structural changes to fetal brain development associated with prenatal maternal stress.
3. Understand the physiological changes that occur in the placenta with maternal stress exposure that (a) protect the fetus from elevated cortisol levels and (b) potentiate the adverse effects of prenatal maternal stress.
4. Recognize the sex-specific and time-specific effects of prenatal maternal stress exposure.
5. Learn about the possible mechanisms that may underlie the effects of prenatal maternal stress.
6. Identify the limitations in current research, and methods to improve future research.

Highlights

Prenatal maternal stress exposure is associated with

- Altered HPA axis function
- Increased risk of affective disorder
- Hyperactivity (ADHD symptomology)
- Cognitive deficits

All outcomes are moderated in a sex-specific and time-of-gestation-dependent manner.

Altered brain structure, cell number, and composition are found in key areas involved in HPA axis regulation, including the limbic system and the prefrontal cortex.

Prenatal exposure to maternal psychosocial stress (prenatal maternal stress) has been associated with increased risk of cardiometabolic disorders, immune dysfunction, as well as neurodevelopmental disorders in affected children [1]. Studies have shown that 15–28% of women report being stressed during pregnancy [2], which refers to maternal distress or anxiety, an acute or chronic event that causes maternal psychosocial distress, or pregnancy-related anxiety.

Stress is a system-wide physiological response that activates both the autonomic nervous and hormonal systems. The sympathetic nervous system (SNS) of the autonomic nervous system becomes the dominant driver of an acute physiological response to stress, such as an increased heart rate, by causing the secretion of epinephrine (adrenaline) from the adrenal medulla. These pathways are augmented by the hormonal system, regulated by the hypothalamus–pituitary–adrenal (HPA) axis, leading to increased secretion of glucocorticoids (GCs) from the adrenal cortex. GCs are a family of stress hormones, where in humans, cortisol is the endogenous form, while corticosterone is the endogenous form in common laboratory rodents, such as mice and rats. In addition to further promoting the immediate responses facilitated by the SNS, GCs also target the metabolic system to regulate blood glucose levels and cause immune imbalances [3].

When a pregnant mother experiences a stressful event during pregnancy, it is both her own body and that of the developing fetus which are affected. High levels of GCs are able to pass through the placenta and affect various aspects

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of fetal development, such as the brain, thus programming the fetus for an altered growth trajectory and other adverse health outcomes.

Neurodevelopment in humans takes place over many years and is a process that is highly sensitive to the environment. Prenatal phases of neurodevelopment include neurogenesis, neuronal migration, synaptogenesis, and gliogenesis, which together form the neural networks and connectomes that will define many aspects of behaviour and potential risk of neurodevelopmental disorders. Although these processes are largely regulated by genomic factors, considerable evidence suggests that fetal brain development is highly influenced by extrinsic factors, such as maternal nutrition, maternal mental health, and environmental factors. As such, sub-optimal environments can place children on trajectories towards poor neurodevelopmental and mental health outcomes. Understanding the nature of these interactions with neurodevelopment and the mechanisms involved have the potential to ameliorate, prevent, or reverse the effects of early adversity. In this chapter, we will focus on the impact of prenatal maternal stress on neurodevelopment to examine the various outcomes that have been observed in exposed children and discuss some of the most recent investigations on the potential mechanisms behind these associations.

Outcomes

Maternal stress has been shown to alter fetal neurodevelopment and lead to long-term behavioural changes in offspring. Further, a number of neuropsychiatric pathologies have been associated with maternal adversity during pregnancy, with offspring demonstrating increased risk of anxiety or depression, motor-sensory deficits, and acquiring neurological disorders, such as schizophrenia or autism [4–11]. Conversely, early postnatal positive reinforcements, such as increased levels of maternal care [12, 13] or being breastfed for over 3 months, can improve neurodevelopmental outcomes [14, 15], indicating opportunity for effective early interventions. Behavioural outcomes in children associated with prenatal maternal stress can be categorized into four broad types; [1] altered HPA axis function (basal levels and stress response) [2] symptoms of hyperactivity (ADHD) [3] symptoms of depression or anxiety affective disorders, and [4] neurocognitive impairments.

Altered HPA Axis Function

The most consistent phenotype observed in offspring following prenatal maternal stress exposure is an altered HPA axis function. The HPA axis controls the stress response via production and secretion of GCs, which in humans, sheep, primates, and guinea pigs is cortisol, and corticosterone in rats and mice. Stress activates the paraventricular nucleus (PVN) of the hypothalamus to produce and release corticotropin-releasing hormone (CRH) into the hypophyseal portal system to stimulate the production of adrenocorticotropin-releasing hormone (ACTH) by the anterior pituitary. ACTH then stimulates the synthesis and release of GC by the adrenal cortex. The majority of circulating GC is inactive due to binding to corticosteroid binding globulin (CBG). In the brain, GCs bind to mineralocorticoid (MR) or glucocorticoid receptors (GRs). MRs, due to their high affinity, bind to GCs at low, basal concentrations, while GRs bind to GCs at higher concentrations during stress-activated states.

Various regions of the brain express GRs and regulate GC feedback pathways. The hypothalamus and pituitary, as well as the prefrontal cortex (PFC) and hippocampus, participate in negative feedback. On the contrary, GR binding in the amygdala stimulates CRH neuron activity in the PVN, thus participating in positive feedback [16]. MR and GR expressions have been identified in these regions from very early in gestation, indicating potential sensitivity of the brain to increased GCs during early development.

In humans, basal and stress-activated HPA function have been shown to be elevated in infants and children born to mothers who experienced stress during pregnancy [17, 18], often demonstrating stronger associations in girls. Sex-specific outcomes are frequently observed following developmental adversities. For example, girls born to mothers who experienced the 2008 Iowa flood demonstrated a significant increase in cortisol levels at 20 and 45 mintues following maternal separation stressor as compared to baseline at 2.5 years of age [19]. The timing of exposure during gestation also appears to be critical, as in another study, girls born to mothers who reported higher levels of anxiety in mid-gestation demonstrated higher daytime salivary cortisol profiles than unexposed girls [20]; however, there were no differences in HPA function when anxiety occurred at other times in pregnancy.

Studies using animal models have identified similar HPA outcomes to humans with regard to sex and temporal specificity. Alterations in HPA activity following prenatal stress in rats in late gestation have been associated with decreased hippocampal MR and GR expression in offspring at postnatal days (PNDs) 21 and 90. Reduced receptor expression was correlated with an increased corticosterone response to stress in juvenile (PND21) rats, and an extended corticosterone response to stress in adulthood (PND90) [21]; effects were greatest in female offspring. In guinea pigs exposed to prenatal maternal stress, adult male offspring demonstrated increased basal and ACTH-challenged cortisol levels as compared to unexposed offspring [22]. In adult female guinea pig offspring, basal cortisol levels were decreased specifically during the luteal phase, and ACTH-challenged

cortisol levels were decreased when animals were in their estrous cycle [23]. These studies demonstrated critical effects of sex and stage of reproductive cycle when studying HPA axis function in offspring following prenatal stress. In non-human primates, maternal stress in mid- to late pregnancy is associated with elevated basal plasma ACTH and cortisol levels in offspring [24]. In general, human [19, 25, 26] and animal [22, 27–29] studies have indicated that prenatal stress is associated with increased HPA responsiveness in offspring, and that effects are generally greater in female offspring when maternal stress occurred in late gestation, though species differences do exist.

Altered Attention (ADHD Symptomology)

Attention-deficit/hyperactivity disorder (ADHD) is a neurological disorder that affects approximately 8.4% of all children in the U.S. and is observed more prevalently in boys than girls [30]. It is commonly marked by patterns of inattention and/or hyperactivity–impulsivity, which result in social morbidities that may interfere with learning in school-aged children or maintaining interpersonal relationships. Symptoms can begin to emerge between 3–6 years (early onset) and 7–12 years (late onset), and decline with age or exhibit lifelong maintenance [31, 32].

During preschool years, children most commonly demonstrate hyperactivity and impulsivity, characterized by constant restlessness and desire for immediate rewards. In elementary school years, inattention can result in educational disruption, while ADHD symptomology in adolescents and adults is further associated with inattention and impulsivity, impeding the development of relationships and resulting in anti-social behaviours [33].

In humans, ADHD phenotypes are commonly observed in association with prenatal stress, which can include general psychological stress [34, 35], depression [36], anxiety [37], and overall lower life satisfaction. As with overall ADHD prevalence, the associations between maternal stress and offspring ADHD are more frequently observed in boys, even after correction for possible confounders, such as prematurity and high-risk pregnancy [36].

In animal models, ADHD-like behaviour can be characterized by examining individual symptoms of hyperactivity or attention. In a guinea pig model of acute maternal stress (gestational days (GDs) 50/51/52), juvenile male offspring exhibited hyperactivity in an open field [22], whereas in adulthood, both male and female offspring displayed reduced activity in the open field [23, 38]. In contrast, male guinea pig offspring born to mothers exposed to chronic stress in late pregnancy (GDs 32–66) demonstrated reduced activity as juveniles [39] but hyperactivity as adults [40], with no effect in females. This highlights the temporal sensitivity of fetal programming events and emphasizes the necessity to further delineate the mechanisms underlying the effect of stress exposure on neurodevelopment. Studies have implicated a role of the placenta in sex-specific programming. In a mouse model of early prenatal stress, stress-induced hyperactivity in adult male offspring [41] was associated with increased placental expression of pro-inflammatory cytokines IL-6 and IL-1®, while no effects were observed in females. Interestingly, these altered behaviours were correlated with decreased expression of dopamine receptors D1 and D2 in the nucleus accumbens of affected male offspring [41], providing insight into the neural signalling pathways that may underlie behavioural differences.

Affective Disorder (Internalizing/Externalizing Behaviour (Depression/Anxiety))

Affective disorders refer to a broad range of internalizing and externalizing behaviours that modulate an individual's social interactions. Individuals with high (positive) affectivity are characterized for their positive attitude and sociability, while negative affectivity includes symptoms of sadness, lethargy, or worry [42]. Internalizing affective disorders, such as depression, anxiety, and post-traumatic stress disorder, are more prevalent in women compared to men, across numerous types of disorders [43], though symptoms are less clear in prepubertal individuals. In children, internalizing behaviours can be characterized as being nervous or irritable, shy, afraid, sad, or lonely, while externalizing behaviours are more vocal and can be disruptive to others, such as defiant or conduct disorders [44]. Externalizing behaviours are more commonly observed with affective disorders in children and demonstrate higher prevalence amongst boys than girls [45]. Negative or positive affectivity can be measured by caregiver reports, such as degrees of depressive- and anxiety-like behaviours, laboratory observations, or cortisol response to stressors.

In humans, affective disorder associated with prenatal maternal stress was highly sensitive to the time of exposure. Prenatal maternal stress in early to mid-gestation was associated with increased externalizing behaviour in children [37, 46] and increased emotional reactivity/temperament in preschool-aged girls, but not boys [47]. Conversely, similar exposures in late gestation extending into the postpartum period were associated with internalizing behaviours and anxiety, an effect which was more strongly observed in infant girls than boys [48, 49].

In young girls (7 years), but not boys, increased internalizing behaviour associated with elevated maternal cortisol during pregnancy was linked to larger right amygdala volume [50], a region involved in stress monitoring and fear processing. Furthermore, higher levels of maternal cortisol

during pregnancy were associated with internalizing behaviour in girls at 2 years, but not boys [51]. This was associated with increased functional connectivity between the amygdala and brain regions involved in sensory processing and integration. Altered affectivity was also observed in association with reduced levels of maternal care, again, more strongly in girls than boys [49]. It is interesting to note that while the prevalence of affective disorders is reported to be higher in boys in population-based studies, girls appear to demonstrate a higher vulnerability to behavioural changes following prenatal stress exposure.

A number of animal studies have been undertaken. Male and female rats born to mothers exposed to mild stress throughout gestation demonstrated anxiety- and depressive-like behaviours, which were correlated with increased basal and activated salivary GC concentrations [52]. Similarly, juvenile male guinea pigs exposed to prenatal maternal stress in late gestation demonstrated increased anxiety-like behaviours [22]. In another study examining a longer period of prenatal maternal stress in guinea pigs, juvenile male and female offspring demonstrated anxiety-like and neophobic behaviour [53], which was associated with reduced hippocampal myelin binding protein (MBP) and glial fibrillary acidic protein (GFAP) expression. Interestingly, analysis of fetal brains of the same cohort identified alterations in MBP and GFAP expression in male animals only [54], suggesting an interaction between sex and developmental timing. These studies highlight the importance of the period and duration of prenatal stress exposure, as well as sex-specific trajectories in the prenatal programming of affective disorder.

Cognitive Impairment

There is considerable literature linking exposure to prenatal stress and poor cognitive outcomes in offspring. In humans, maternal stress during the 1998 Ice storm in Quebec, Canada, was associated with lower intellectual and language abilities in toddlers, especially if exposure occurred during early gestation [4]. Pregnancy-associated anxiety throughout gestation has been linked to decreased visuospatial working memory in school-aged children (both boys and girls) [55]. Elevated maternal cortisol early and late in gestation has been associated with neurodevelopmental delay over the first year of life [56], and poor mental and motor developmental outcomes in infancy [57], respectively. Interestingly, another study showed that high levels of cortisol in amniotic fluid during mid-gestation predicted negative cognitive ability in infancy, though this association was only significant in infants with low maternal attachment [13]. The profound interaction of prenatal stress and postnatal environment may explain why in some studies, maternal distress was associated with enhanced cognitive development [56, 58]. Such sensitivity to maternal care indicates that developmental trajectories continue to be shaped postnatally further indicating opportunity for positive intervention.

Impaired memory and learning have been associated with prenatal stress in both young and adult rat offspring, with larger effects in males [59–64]. These impairments have been associated with reduced long-term potentiation in the CA1 region of the hippocampus in rats [60] and mice [65], possibly mediated by the reduced expression of the NR1 and NR2B subunits of the NMDA receptor [65]. Altered NMDAR expression in the hippocampus may be a strong early biomarker of postnatal cognitive impairments, as decreased NR1 and increased NR2A expression have been observed in fetal hippocampus of female guinea pigs following exposure to synthetic GCs [66]. Furthermore, early postnatal stress was associated with decreased NR2A expression in the adult rat hippocampus [67, 68].

The role of the sigma1 receptor has been investigated in elucidating the link between prenatal stress and reduced memory and learning in rats [69]. The sigma1 receptor is integral to an intracellular system which amplifies cellular response to neurotransmitter signals [70]. In adult rats born to mothers who had experienced stress during pregnancy, impaired working memory associated with the prenatal stress was prevented by pre-treatment with a sigma1 receptor agonist prior to memory testing, suggesting that following maternal stress, cells involved in learning may not be as excitatory as in control animals. Amelioration of symptoms with an agonist pre-treatment indicates a causal role of the sigma1 receptor in memory and learning in rats. Other studies have shown prenatal stress in rats to result in poor performance in hippocampal-related spatial tasks [71], effects that were associated with reduced learning-induced neurogenesis in the hippocampus. Reduced neurogenesis following prenatal stress may increase vulnerabilities, particularly in aging, as decreased memory performance in aged, but not young adult rats has been observed following prenatal stress [72]. It is possible that altered neurogenesis during critical developmental windows may lead to differences in brain structure and connectivity, resulting in adverse phenotypes with lifelong consequences.

Mechanisms

The mechanisms by which early adversity impacts neurodevelopmental and behavioural outcomes in offspring are an area of intensive investigation. Increased knowledge in this area may enable the development of interventions that ameliorate, prevent, or reverse the longer-term consequences of adversity. A number of studies have demonstrated that maternal adversity is associated with altered brain structure, serotonin production, maternal and fetal immune activation,

placental dysregulation, as well as genomic and epigenomic changes. It is most likely that multiple drivers are involved and that no one pathway independently regulates phenotypic outcomes.

Brain Structure

Glucocorticoid exposure decreases cell proliferation and neuronal differentiation in the fetal brain [73]. Elevated maternal cortisol levels during pregnancy have been associated with reduced fetal brain growth [74], while maternal depression in early to mid-gestation has been associated with cortical thinning in the right inferior frontal cortex and middle temporal cortices [46], indicating vulnerabilities in structural growth of the brain following maternal stress.

The right amygdala is responsible for negative emotional processing. Increased right amygdala volume has been consistently identified, particularly in girls born to mothers who experienced psychological stress during pregnancy [50, 75] and in a population of girls raised in orphanages [76]. These structural changes were associated with negative affectivity, increased anxiety, and internalizing behaviour [50]. While most structural studies have been undertaken in children, a study has reported a reduction in right amygdala grey matter volume in women born to mothers who experienced stress in pregnancy [77], indicating persistent long-term structural consequences associated with prenatal adversity. It has been reported that neurodevelopment occurs more rapidly in female fetuses than male fetuses [78, 79], suggesting a potential route by which sex differences may be established in response to maternal stress in pregnancy [78, 79] or high levels of GCs.

In humans, robust associations between prenatal stress and increased functional connectivity between the right amygdala and cortical brain regions have been identified in girls, but not boys [80]. However, in a different study, decreased white matter integrity and connectivity between the right amygdala and the corticostriatal circuitry were observed in association with prenatal stress exposure [81]. Connections to the PFC (dorsolateral and right ventral PFC), insular, and temporal cortices were most significantly reduced, alterations in which were associated with internalizing behaviour [82]. It is possible that the differences in white matter structure are modulated by differential expression of GC-sensitive genes involved in gliogenesis, which are known to be expressed in the developing amygdala [83]. In a rat model of prenatal stress, enlarged lateral amygdaloid nuclei were observed due to hypertrophy of neurons and glial cells [84], and reduced white matter volume was observed in the PFC following early postnatal stress [85], though only male animals were tested. Together these studies indicate that the amygdala is vulnerable to early adversity resulting in altered cell composition, structure, and connectivity to other brain regions.

In the rhesus monkey, prenatal stress resulted in offspring with reduced hippocampal volume and reduced neurogenesis in the dentate gyrus [86]. In rat hippocampi, prenatal maternal stress was associated with atrophy of pyramidal neurons in the CA3 regions and decreased glial cell count in the pyramidal layer of juvenile females [87], but not males [88]. Decreased length and complexity of dendritic spines in the CA1, CA3, and dentate gyrus of the hippocampus were observed in female and male offspring born to mothers that had been exposed to stress in pregnancy [87, 89, 90]. In additional experiments, decreased GABAergic neurons [91] and increased autophagy [92] were reported, though only males were investigated. In the majority of cases, offspring also exhibited altered behavioural phenotypes, including decreased cognitive flexibility, altered affectivity, and heightened HPA axis activity. Overall, inhibition of learning-induced neurogenesis and decreased levels of lifelong neurogenesis in the dentate gyrus have also been identified in rats following prenatal stress [71]. These structural and functional changes suggest that prenatal stress and GC exposure profoundly alter limbic structures, and this likely underlies a number of the cognitive deficits described.

Serotonin

Serotonin (5-HT) is involved in the development and migration of neurons and synaptogenesis, and altered 5-HT signalling influences neuronal connectivity in the forebrain [93–95]. This likely involves cross-talk with brain-derived neurotrophic factor (BDNF), which is involved in synaptogenesis, as well as interaction with GABAergic, glutamatergic, and dopaminergic systems. Impaired 5-HT signalling has also been linked to psychiatric disorders, including anxiety, depression, ADHD, and schizophrenia [96].

In early gestation, the placenta serves as the primary source of fetal 5-HT, as fetal brain 5-HT systems do not mature until later in gestation and maternal 5-HT does not readily cross the placenta [93]. Maternal tryptophan (TRP), however, enters the placenta and is converted to 5-HT by TRP hydroxylase (TPH) [97] which then passes to the fetal circulation.

Studies in rats have demonstrated increased 5-HT levels in the fetal brain following maternal stress [98–100]. In the mouse, mild inflammation in mid-gestation resulted in increased fetal brain 5-HT, and this was associated with increased placental TRP expression and TPH activity [93], resulting in higher 5-HT output in the umbilical vein [97]. Increased 5-HT in the fetal forebrain was prevented by pharmacological inhibition of TPH1 to suppress placental TPH activity suggesting a causal relationship [97]. It is unclear

how altered fetal brain 5-HT signalling leads to long-term changes in behaviour after birth. However, *in vitro* studies of mice, rat, and guinea pig fetal hippocampal neurons demonstrated that 5-HT modified GR transcription and binding [101–103]. These data clearly suggest a link between prenatal stress, placental regulation of 5-HT production, and subsequent serotonergic signalling in the fetal brain.

Inflammation

Although GCs are widely accepted to have anti-inflammatory properties, they can also potentiate immune function [104]. Psychological stress, especially when chronic, is a potent stimulator of the immune system in the brain and periphery [105, 106]. GCs can promote a pro-inflammatory state by altering leukocyte composition (adaptive immunity), and stimulating expression of cytokines (IL-6, TNF-⟨, IL-1®) [107, 108], transcription factors (NF-lB) [109], prostaglandins (PGE2) [58], and downstream regulatory proteins, such as NLR family pyrin domain containing 3 (NLRP3) [58], which cleave pro-inflammatory cytokines to their mature form. Pro-inflammatory cytokines stimulate HPA function [110, 111].

In humans, maternal stress potentiates a pro-inflammatory milieu by increasing expression of C-reactive protein (CRP; an acute phase inflammatory response protein), pro-inflammatory cytokines and chemokines, while down-regulating expression of anti-inflammatory cytokines, such as IL-4 [112–114]. Animal studies have demonstrated that altered neurobehavioural outcomes in offspring exposed to maternal inflammation are associated with increased inflammation in the fetal brain, characterized by increased pro-inflammatory cytokines and chemokines [107]. Interestingly, NSAID treatment of mice during prenatal stress prevented the behavioural phenotypes in offspring, suggesting a possible causal role of inflammatory signalling in mediating the neurobehavioural outcomes following maternal adversity [41].

Maternal stress in mice has also been shown to stimulate a pro-inflammatory shift in the placenta, resulting in increased cytokines, chemokines, COX proteins, and prostaglandins [41]. Increased placental IL-1® expression has been associated with decreased expression of BDNF in both the placenta and fetal brain [41, 115], indicating potential alterations in signalling pathways regulating synaptogenesis and synaptic plasticity [116]. BDNF dysregulation has been implicated in the pathophysiology of affective behaviour [117], suggesting a potential molecular pathway by which prenatal stress, fetal inflammation, and neurodevelopment are related through the placenta.

Placental 11β-Hydroxysteroid Dehydrogenase 2 (11β-HSD2)

The placenta expresses high levels of 11β-HSD2, an nicotinamide adenine dinucleotide (NAD) dependent enzyme, which converts active (e.g. cortisol and corticosterone) to relatively inactive (e.g. cortisone and 11-dehydrocorticosterone) GC isoforms. This results in a significant gradient in active GC concentrations between the mother and fetus. Fetal plasma GCs are five to ten- fold lower than in the maternal circulation [118]. However, human [119] and mouse [120] studies have demonstrated that there are incremental increases in fetal plasma GC concentrations following maternal stress, which are associated with decreased expression of placental 11β-HSD2 in humans [121, 122] and rodents [123, 124]. These effects appear sex specific, with strongest effects observed in female placentae [17, 125], suggesting reduced protection in female fetuses to prenatal stress exposure.

In a recent mouse study, prenatal stress resulted in increased plasma corticosterone levels in female, but not male fetuses [120]. Interestingly, in males but not female fetuses, prenatal stress increased the placental expression of genes that confer fetal GC protection, such as 11β-HSD2 and ABCB1 transporters, which efflux GCs to the maternal circulation [120]. These findings represent another mechanistic pathway underlying sex differences in neurodevelopmental and behavioural outcomes following maternal stress.

Genetic and Epigenetic Mechanisms

Recently, there has been considerable focus on the role of epigenetic mechanisms in the long-term programming of phenotypes. Epigenetic modifications include DNA methylation, histone modifications, and microRNAs[127] (see Chap. 8). DNA methylation, the most commonly studied modification, is the addition of a methyl group on the cytosine of a cytosine guanine dinucleotide (CpG), which can decrease gene transcription potential, especially if occurring in gene regulatory regions. Histone modifications include a collection of post-translational modifications, including methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation [126]. Together, histone modifications have the potential to alter gene expression by altering chromatin structure and chromatin availability to transcriptional proteins. microRNAs are short non-coding RNA sequences involved in post-transcriptional regulation to target mRNA sequences to degrade or inhibit binding of translation-related enzymes.

During development, epigenetic changes regulate tissue-specific gene expression to promote cellular differentiation.

In mice, genome-wide CpG demethylation occurs in the blastocyst around embryonic day 3.5 (E3.5), after which a period of global re-methylation occurs until around E8.5 [127]. This represents a critical period of high sensitivity to environmental exposures, which may explain why adversity in early pregnancy is associated with stronger phenotypes than later exposures. After E8.5, various tissues, including the brain, continue to develop tissue-specific epigenetic patterns that contribute to cell differentiation and differential gene expression. Tissue-specific methylation signatures may also dictate differential adaptations to environmental stimuli, such as maternal stress.

A number of studies have investigated the impact of prenatal stress on epigenetic marks in offspring, particularly in brain regions that regulate behavioural phenotypes, including the hippocampus, amygdala, and PFC. In mice, genome-wide changes to the hippocampal epigenome have been identified in offspring following maternal stress [128]. Exposure of pregnant guinea pigs to GCs resulted in genome-wide modifications to DNA methylation and H3K9 acetylation in exposed offspring, which were associated with an altered hippocampal gene transcription [129, 130]. In targeted studies, increased DNA methylation and decreased transcription of the *nr3c1* gene, which encodes for GR, have been identified in mice and rat offspring following maternal stress in pregnancy [131, 132]. Maternal exposure to corticosterone during pregnancy in mice resulted in anxiety-like phenotypes in adult offspring, which was associated with decreased *fkbp5* DNA methylation in the hippocampus [133–135]. FKBP5 is a negative regulator of cortisol response by inhibiting nuclear translocation of the glucocorticoid/GR complex. Together, increased methylation of *nr3c1* and decreased methylation of *fkbp5* suggest a reduced responsiveness of the hippocampus to GCs, which would decrease inhibitory effects on the HPA axis resulting in hyperactivity (described above).

Increased DNA methylation in *bdnf* and reduced gene expression in the hippocampus have been reported following prenatal maternal stress in young and adult mice [136, 137] and adult male rat offspring [138]. In mice, reduced hippocampal *bdnf* expression was also associated with altered neurobehavioural outcomes in young offspring, including depression- and anxiety-like phenotypes [136]. Similar profiles of hippocampal *bdnf* expression were observed in adult mice and rats following early postnatal stressors, including low maternal care or predator exposure, respectively [139–141]. Sex-specific effects have also been reported where hippocampal *bdnf* expression was reduced in adult female mice offspring following in prenatal maternal stress, but not in males [142].

BDNF cross-talk with the glutamatergic and GABAergic systems during development has been well established [143]. Indeed, altered *bdnf* expression in the hippocampus of rats born to mothers exposed to prenatal stress was associated with increased *nr2b* expression [144], which encodes for the NMDAR subunit 2b. In another study, restraint stress during pregnancy resulted in increased DNA methylation in *reelin* and *gad67* (glutamate decarboxylase, which converts glutamate to GABA) promoters and decreased mRNA levels in the hippocampus and PFC of adult male offspring [145], which were associated with hyperactivity, reduced social interaction, and impairments in fear conditioning. The behavioural profiles are comparable to phenotypes of schizophrenia, and similar changes in DNA methylation have been observed in post-mortem brains of patients with schizophrenia, highlighting the potential clinical importance of DNA methylation in glutamatergic and GABA regulatory genes [145].

Decreased expression and increased DNA methylation of *bdnf* regulatory regions has been observed in the PFC of adult male mice exposed to prenatal stress (females were not investigated) [137], and similar changes have been reported in both male and female adult rats exposed to early postnatal stress [146]. However, effects of prenatal stress on DNA methylation on *bdnf* regulation in the amygdala have been more variable, with some studies reporting increased methylation and decreased expression in adult male rats (females not investigated) [138], and others observing decreased methylation in adult female mice following prenatal maternal stress with no effects in males [142]. Another study reported no change in *bdnf* methylation in the adult rat amygdala following early postnatal stressors [141]. The variability in findings likely indicates epigenetic specificity to fetal sex, species, and the time in development when the stress was experienced.

Sex-specific expression of the placental *ogt* gene may be one mechanism by which epigenetic specificity may be established. O-linked N-acetylglucosamine transferase (OGT) is a GC-sensitive, X-linked gene that is expressed at nearly two-fold higher levels in female mouse placenta compared to the male placenta [147]. Genetically manipulated females hemizygous for placental *ogt* (X^{WT}/X^{ogt-}) exposed to prenatal maternal stress demonstrated phenotypic and genotypic resemblance to wild-type male offspring. This included a hyperactive HPA response to stress in adulthood [147, 148] and an altered fetal hypothalamic transcription profile, which included genes involved in sexual differentiation, sexual responsivity, and neurodevelopmental disorders [149]. Furthermore, placental H3K27me3 patterns of hemizygous female fetuses were significantly reduced, producing similar patterns to male placenta [149]. It is plausible that OGT regulation of sex-specific differences in H3K27me3 may be one mechanism by which sex-specific phenotypes are established in mice following prenatal stress exposure. Indeed, female

mice offspring with placenta-specific decreases in H3K27me3 were more vulnerable to hypothalamic gene expression changes following prenatal stress as compared to wild-type females (high placental H3K27me3) [149], indicating an important role of the epigenomic landscape in the programming of long-lasting phenotypic changes following prenatal stress.

Paternal Influence

Recent evidence indicates a powerful paternal influence on neurodevelopmental outcomes in offspring. Lower basal cortisol levels, blunted HPA axis response to dexamethasone treatment, and an increased prevalence of neuropsychiatric disorders, such as PTSD and depression, have been reported in the adult male offspring of male Holocaust survivors [150, 151]. Similarly, mouse models of paternal preconception stress consistently demonstrate altered HPA function, including reduced HPA responsiveness to stress [152, 153] in both male and female offspring [154].

It is hypothesized that paternal transmission of stress occurs through epigenetic changes in the sperm and seminal fluid, possibly including DNA methylation, miRNA and tRNA. Indeed, DNA methylation of stress-regulated genes were altered in the sperm of male mice exposed to stress prior to mating [155]. Interestingly, alterations in DNA methylation were found in the same stress regulatory genes in the brains of the mice offspring, that also displayed depressive-like phenotypes [155]. At the level of miRNA, paternal stress prior to mating has been associated with increased levels of 9 miRNAs in sperm [153]. In this model, paternal stress resulted in a blunted HPA responsiveness to stress in the offspring [153], a phenotype which could be recapitulated by microinjection of the 9 miRNAs into 'control' zygotes [156]. Through this set of elegant experiments, the causal role of the miRNA was established in a mouse model preconception paternal stress.

The impact of paternal stress and the mechanisms by which the effects are transferred to the developing fetus is a new area of research that is being investigated. The complex pathways by which the environment influences the epigenome and subsequently leads to altered behavioural phenotypes in offspring remains to be elucidated. It will be important in future studies to consider both maternal and paternal influences to fetal development, examining various mechanisms in concert, including changes to the DNA methylome and miRNA expression, with potential confounding effects from intrinsic genomic sequences, such as SNPs.

Conclusion

Maternal adversity in pregnancy is associated with altered neurodevelopmental outcomes in a sex-specific manner. Female offspring demonstrate increased vulnerability to neuroendocrine dysfunction and affective disorders, such as internalizing behaviours, depression, and anxiety, while male offspring are more likely to be susceptible to neurocognitive impairments in learning and memory, primarily in male offspring. A wide variety of outcomes may be due to alterations in brain structures, including the amygdala, hippocampus, and PFC and a modified connectome. The temporal sequence of processes in brain development may dictate critical windows of development during which maternal stress can lead to differential behavioural outcomes. Placental production of factors, including serotonin, which are critical to shaping neuronal networks in brain regions, such as the PFC, may be particularly susceptible to maternal stress and underlie many of the sex differences that are commonly observed in behavioural phenotypes. Epigenetic mechanisms, such as DNA methylation and altered miRNA expression, also likely represent an important link between early exposure and long-term outcome. Future mechanistic studies examining the combined contribution of genotype and various epigenetic markers, in the context of placental function, immune homeostasis, and neurodevelopmental processes will be imperative in elucidating the associations between stress and long-term neuroendocrine, neurobehavioural, and neurocognitive outcomes. Improved knowledge of the mechanisms involved will allow development of interventions that protect the developing brain against the impact of early adverse exposures.

End-of-Chapter Summary

Prenatal maternal stress can shape the fetal neurodevelopmental trajectory to alter postnatal behaviour and neuroendocrine phenotypes. Although studies to date clearly indicate that there is an association between the prenatal environment and neurodevelopment, more work needs to be done to delineate the intricacies behind sex differences and the differential effects of exposure during critical periods of development. Through this chapter, you will have learned about some of the outcomes following prenatal maternal stress exposure, and possible mechanisms that underlie these associations. Altered placental function, maternal inflammation, as well as epigenetic changes likely play a role in driving altered neurodevelopmental trajectories associated with maternal stress. Most recently it has become evident that the preconception mental

health of mothers and fathers may also shape fetal neurodevelopment. This highlights the importance of supporting mental health, before, during, and after pregnancy. Understanding the various outcomes, and the possible mechanisms involved, will assist in the development of effective and appropriately timed interventions aimed at ameliorating, preventing, or reversing the long-term impact of prenatal maternal stress.

Multiple-Choice Questions (5)

Q1. Which one of the following offspring outcomes following prenatal maternal stress exposure is correct?
- (a) In the 1998 Ice Storm cohort, lower intellectual abilities were found in toddlers, especially if exposure occurred during mid-gestation.
- (b) Prenatal maternal stress exposure is associated with higher levels of hippocampal myelin binding protein in both male and female guinea pigs, although anxiety-like phenotypes were only observed in female offspring.
- (c) **Prenatal maternal stress is associated with an elevated cytokine levels in the maternal circulation and placenta, which can lead to hyperactivity in adult male offspring, but not in adult females.**
- (d) In adult male guinea pigs exposed to prenatal maternal stress, an increased basal cortisol level was detected, but further stimulus with ACTH did not show any differences in HPA axis response as compared to unexposed offspring.
- (e) All statements are incorrect.

Q2. *Fkbp5*, a negative regulator of cortisol response, shows (a) DNA methylation in mouse hippocampus, associated with (b) anxiety-like phenotypes in adult offspring following prenatal maternal corticosterone exposure.
- (a) (a) increased; (b) increased
- (b) (a) increased; (b) suppressed
- (c) **(a) decreased; (b) increased**
- (d) (a) decreased; (b) suppressed
- (e) (a) unchanged; (b) increased

Q3. Which one of the following statements concerning prenatal maternal stress is incorrect:
- (a) Reduced connections to the prefrontal cortex were found in association with prenatal maternal stress exposure.
- (b) Hypertrophic neurons and glial cells are responsible for an enlarged amygdaloid nucleus in rat brains following prenatal maternal stress.
- (c) **Prenatal maternal stress was associated with more hippocampal neurons in the CA1, and CA3 regions, though axon lengths were shorter.**
- (d) Internalizing behaviour observed in girls exposed to prenatal maternal stress was associated with an increased right amygdala volume.
- (e) All of the above are incorrect.

Q4. Which one of the following statements regarding genetically modified female hemizygote mice for the placental *ogt* gene (X^{WT}/X^{ogt-}) is correct:
- (a) Genetically identical to male knock-out mice
- (b) **Hemizygous female mice demonstrate elevated cortisol response to stress in adulthood**
- (c) Placental ogt regulates hypothalamic DNA methylation of glucocorticoid response elements
- (d) Ogt is a glucocorticoid-sensitive gene that regulates placental metabolism of tryptophan into serotonin
- (e) All of the above are correct.

Q5. **True** /False: In mice, 9 specific sperm miRNAs were found to demonstrate a causative link between paternal preconception stress and blunted HPA responsiveness to stress in the offspring.

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Sex Differences in Neurodevelopment and Its Disorders

11

Richard E. Brown

Learning Objectives

- The role of genes in sex determination.
- The role of hormones in sexual differentiation.
- That sex differences develop during critical periods of development.
- That genes and hormones are involved in the sexual differentiation of the brain
- That there is a sexual differentiation of glial cells (astrocytes and microglia) and that these cells are involved in the sex differentiation of the brain and neuroendocrine system.
- That there are sex differences in brain structure, function and neurochemical pathways that underlie sex differences in NDD.
- That the sexual differentiation of the hypothalamic-pituitary (neuroendocrine) system results in sex differences in the secretion and actions of hormones throughout the body.
- That hormones may underlie the sex differences in NDD disorders.
- That puberty is a critical period for the reorganization of brain and neuroendocrine systems during the transition from childhood to adulthood.
- That neurotoxins and endocrine-disrupting chemicals in the environment can disrupt sexual differentiation during critical periods of development.

Highlights

- Sex differences depend on the XX and XY chromosomes.
- Sex differences depend on the gonadal steroid hormones, estrogen, and testosterone.
- Sex differences depend on the sexual differentiation of neurons and neurotransmitter pathways in the hypothalamus and related brain areas: the amygdala, hippocampus, and arcuate nucleus.
- Sex differences depend on glial cells and gliotransmitters.

Introduction to Sex Differences in Neurodevelopmental Disorders

Anatomical, physiological, and cognitive-behavioral systems in mammalian development all show some form of sexual dimorphism [1–3]. Sex differences also occur in susceptibility to disease [4], and among neurological diseases, "virtually every neurodegenerative and neuropsychiatric disease shows some variation, often striking, between males and females" ([5], page 2). Zagni, et al. [6] noted that NDDs occur more often in males, while adult-onset neurological disorders have a higher frequency in females. Neuroendocrine abnormalities may underlie a number of NDDs [7, 8] and this chapter examines the genetic, epigenetic, and neurochemical factors that underlie sex differences in NDDs.

Table 11.1 lists some NDDs, giving estimates of their frequency of occurrence per 100 children [9] and the sex ratio of children with each disorder [10, 11]. In many NDD, the sex ratio may not differ, but the neural and behavioral manifestations of the disorder show sex differences [12, 13]. This chapter describes the process of sex determination and then examines the role of genes, hormones, glial cells, and neuroimmune interactions in sexual differentiation of the brain and nervous system and how these mechanisms regulate sex

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Table 11.1 List of NDD, prevalence, and sex ratios

Disorder	Prevalence per 100 (%)	Sex Difference (M:F)	Reference
ADHD	5.0	2.1–3.0:1.0 (M>F)	[11, 365, 366]
Angelman Syndrome	0.0040	1.0: 1.0 (M=F)	[367]
Asperger syndrome	0.005	4-6:1 (M>F)	[366]
Autism Spectrum Disorder	0.65	4.0:1.0 (M>F)	[11, 365, 366]
CDKL5		1.0:5.0 (F>M)	[367]
Cerebral Palsy	0.15	1.0:1.0 (M=F)	[10]
Cornella de Lange Syndrome	0.0014	?	[10]
Cri du Chat Syndrome	0.0020	(F>M)	[10]
Depression & Anxiety		1.0:2.0 (F>M)	[11]
Developmental coordination disorder	6.5		[368]
Developmental dyscalculia	3.0	?	
Developmental dyslexia	6.0	?	
Developmental intellectual disability	0.004-0.0300	1.0:1.0 (M=F)	[366]
Down syndrome	0.1667		[10]
Duchenne muscular dystrophy	0.0143		[10]
Fetal Alcohol syndrome	0.1000	1.3:1,0 (M>F)	[10, 369]
Fragile X syndrome	0.0615	2.0:1.0 (M>F)	[10]
Galactosemia	0.0020	?	[10]
Intellectual disability	5.5	1.6:1.0 (M>F)	[365]
Klinefelter syndrome (XXY)	0.086	Only males	[10]
Language disorders		M>F	[370]
Lesch-Nyhan syndrome	0.0005		[10]
Lowe syndrome	0.0005		[10]
Marfan syndrome	0.0067	M=F	[10, 367]
Motor disorders	3-6%		[365]
Multiple sclerosis		1.0:2.0–3.0 (F>M)	
Neurofibromatosis Type 1	0.0308		[10]
Noonan syndrome	0.0571	M>F	[10, 367]
Phenylketonuria	0.0100	?	[10]
Prader-Willi Syndrome	0.0067	M=F	[10, 367]
Rett Syndrome	0.0080	Only F	[10, 367]
Rubinstein-Taybi Syndrome	0.0008		[10]
Specific language impairment	7.4	M>F	[370]
Specific Learning Disorder	5–15	2.1–3.1/1.0 (M>F)	[365]
Tic disorders	.3%	2.1–4:1.0 (M>F)	[365]
Tourette Syndrome	0.5000	M & F differ in Symptoms	[10, 371]
Tuberous sclerosis	0.0167	M=F	[10, 367]
Turner Syndrome (45XO)	0.0400	F only	[10]
Trisomy 18	0.0250		[10]
Chromosome 22q11.2 Deletion Syndrome			
Velocardiofacial syndrome	0.0250		[10]
Williams Syndrome	0.0044	M=F	[10, 367]
XYY syndrome	0.0545	Only males	[10, 367]
Trisomy X(XXX)	0.0550	Only females	[10, 367]

differences in the development of NDD. The chapter concludes with a discussion of sex differences in NDD at puberty and a discussion of the theories about the causes of sex differences in NDDs.

Sex Determination and Sexual Differentiation

In mammals, sex determination depends on the genes on the sex chromosomes, while sexual differentiation depends on the action of gonadal hormones and other chemical signals, which activate gene expression differentially in each sex. Females have two X chromosomes (46 XX in humans; 40 XX in mice), while males have an X and a Y chromosome (46XY in humans; 40XY in mice). Most of the research has been done on rodents and the ability to make genetically modified mice has enabled sex differences determined by genes and those determined by hormones to be dissociated [3, 14–19].

Masculinization depends on the *SRY* ("sex-determining region Y") genes on the TDF (testis-determining factor) locus on the Y chromosome [20–25]. Early in embryonic development, the gonads are undifferentiated and are identi-

cal in males and females, having bipotential precursor cells which can develop either into testis or ovaries. The differentiation of the bipotential gonad into a testes is determined by the *SRY* genes on the Y chromosome and is controlled by a cascade of gene expression which is regulated by transcription factors. The differentiation of these precursor cells into testes is induced by the expression of the *SRY* gene in somatic cells that differentiate into Leydig (Androgen producing) and Sertoli (sperm producing) cells.

If there is no Y chromosome or if the *SRY* gene is missing, the bipotential precursor cells of the gonad develop into ovarian follicular cells and, under female-specific gene expression, oocytes develop. If there is a Y chromosome or if an X chromosome has *SRY* gene, testes develop. At the beginning of embryonic development, embryos of the two sexes differ only by their sex chromosomes, but once the Sertoli cells begin to secrete androgens, sexual differentiation begins. The *DMRT1* gene is essential for maintaining the differentiated testes and for preventing ovarian development in postnatal mouse testes [21, 26]. The Y chromosome may therefore act independently of sex hormones to regulate sex differences in growth and metabolism, cardiovascular diseases, immune system physiology, and autoimmune and infectious diseases [16, 27]. The testes-determining gene *SRY* is also expressed in the hypothalamus, midbrain, and cortex and masculinizes these neurons. *SRY* is particularly expressed in the Tyrosine hydroxylase (TH) containing dopaminergic neurons of the Substantia nigra pars compacta (SNpc) that project to the striatum and masculinizes these neurons which are involved in motor behavior [28]. Downregulation of *SRY* in these TH neurons in the substantia nigra (SN) results in deficits in DA and in sensori-motor function (as occurs in Parkinson's disease, which occurs more often in males than females) [28].

There is no single ovary-determining gene in females that is analogous to *SRY* in males, but there are a number of genes and transcription factors that activate ovarian development in the absence of *SRY*. Throughout the development of the ovary, the *FOXL2* gene is required to suppress *DMRT1* expression and prevent the masculinization of granulosa and theca cells into testicular Sertoli and Leydig cells [21, 26]. The granulosa cells of the ovary produce the female sex steroid hormones, estrogen and progesterone, and the follicular cells produce the oocytes [20, 25, 29].

Although females have two X chromosomes, one is inactivated in each cell during development, so that females are mosaics, with some cells having a maternal X chromosome (Xm) and some cells having a paternal (Xp) chromosome [3]. Thus there is a sex difference in X chromosome "dosage": females have two X chromosomes and males only one [30]. There are three X chromosome effects that influence sexual differentiation: (1) The X chromosome that is inactivated can be from the mother (Xm) or from the father (Xp) resulting in "*parental imprinting*" or parent of origin effects as the active X chromosome expresses the trait of the parent from which it was derived ("you have your mother's eyes, but your father's nose"); (2) Some genes *escape from X inactivation* and are expressed on both X chromosomes in females; and (3) Some genes are "dosage dependent" and cells with two X chromosomes may express more of these genes than cells with one X chromosome [30]. Genes on the X chromosomes appear to influence sex differentiation of females, but the expression of genes on the X chromosomes is a complex phenomenon.

Genetic and Epigenetic Disorders of Sex Determination

A number of NDDs are due to abnormalities in sex chromosomes and sex determination [31] (Table 11.2). These disorders are the result of abnormalities in gene transcription during the differentiation of the bipotential gonad into testes or ovaries [32, 33]. In Turner's syndrome, a female has only one X chromosome (45X0) and is incompletely feminized, with gonadal dysgenesis, premature loss of oocytes, and infertility. In Klinefelter's syndrome, the most common disorder of male sex determination, a male has one or more

Table 11.2 Disorders of sex determination and sex differentiation and the five sexes [26, 34, 296]

A. Disorders of sexual determination: Genetic disorders	
46XX	Normal female
45X0	Turner's syndrome (female) 1:2000 girls
47XXX	Trisomy X (female) 1:1000 girls
$46XX^{Y}$	Masculinized female or XX male syndrome (male) 1:25,000 males
47XXY	Klinefelter Syndrome (males) 1:600 births
48XXYY	48 XXYY syndrome (male) 1: 18,000 males
47XYY	XYY syndrome (male) 1:1000 males
46XY	Normal male
B. Disorders of Sexual differentiation: Hormonal disorders	
Female: Ovaries and vagina	
Masculinized females: Congenital adrenal hyperplasia: Ovaries and Penis	
Hermaphrodite: Ovaries & testes; Penis and vagina	
Feminized male: Congenital androgen insensitivity syndrome: testes and vagina	
Kallmann syndrome: Failure of the development on the GnRH neurons: hypogonadotropic hypogonadism (HH) with anosmia	
Females that are masculinized at puberty: 5alpha-reductase 2 deficiency: testes	
Male: testes and penis.	
C. The Five sexes	
Male	
Male pseudohermaphrodite	
True hermaphrodite	
Female pseudohermaphrodite	
Female	

extra X chromosomes (47XXY; 48XXXY) and is partially feminized, resulting in small testes, infertility, and the possibility of ambiguous external genitalia at birth [26, 34, 31]. Trisomy X (47XXX) is the most common female chromosomal abnormality and has little effect on sexual differentiation [35]. If the *SRY* gene is displaced from the Y to the X chromosome, then a person with a $46XX^{SRY}$ phenotype is masculinized and develops male genitals. Because there is a "normal" male phenotype, this disorder is often not discovered until puberty is delayed or men are found to be infertile or develop gynecomastia. However, the testes may be small and there may be incomplete masculinization of the external genitals at birth [36]. Males with 47XYY syndrome have a normal male phenotype but may have reduced fertility [37]. In 48XXYY syndrome males have both an extra Y and an extra X chromosome, resulting in hypogonadism and reduced fertility [38]. In the majority of these sex chromosome disorders, there are neurobehavioral developmental disorders, which are described in the references cited.

The differentiation of the bipotential gonad into testes or ovaries requires epigenetic mechanisms that regulate the temporally organized sequence of gene expression for both sex determination and sexual differentiation [39]. DNA methylation is essential for X chromosome inactivation and for genomic imprinting and epigenitic dysfunction can disrupt these processes in developing females. Disorders of DNA methylation may also disrupt the ability of the *SRY* gene to masculinize males [40]. Likewise, disruption of histone methylation and noncoding RNA functions can disrupt sex determination [39]. Environmental stimuli, including nutrients and endocrine disruptors, can also disrupt sex determination and sexual differentiation [41].

The Neurobiology of X-Linked Intellectual Disabilities and Infectious Diseases

Over 140 genes on the X chromosome may contribute to X-linked intellectual disabilities [42] and other X-linked genes contribute to immune system function and susceptibility to infectious diseases [43]. Because males have only one X chromosome, many X-linked intellectual disorders affect males more than females; however, some of these, such as fragile X syndrome, affect both sexes. Others, such as Rett syndrome (RTT) and CDKL5 syndrome, occur almost exclusively in females [44, 45] but do occur in some males [46–48]. Sex hormones interact with genetic and epigenetic factors in determining sex differences in NDD and this interaction will be discussed in Section "Genetic and epigenetic factors in brain sexual differentiation". Mutations in X-linked genes involve loss of neuronal function by altering dendritic spine size, shape, and density, resulting in the impairment of both excitatory and inhibitory neurotransmission [49]. The synaptic pathologies related to Fragile X syndrome are caused by mutations in the Fmr1 gene; RTT, caused by Mecp2 gene mutations; and "atypical Rett syndrome," caused by CDKL5 mutations [49]. X-linked NDD can also be caused by mutations in Rgo GTPase genes and genes coding for cell adhesion proteins, such as L1CAM and the Neuroligins, all of which affect synapse function [49].

Human females have a stronger innate and humoral immune response than males and are, therefore, less susceptible to many infectious diseases (bacterial, fungal, parasitic, and viral infections) but more prone to autoimmune disorders [43, 50, 51]. These sex differences in immune responses (Fig. 11.1) may be due to X chromosome-linked genes and to X chromosome inactivation as the noncoding micro RNAs on the X chromosome can influence the sex bias in disease frequency [43, 51]. As a result of sex differences in the immune system, there are also sex differences in responses to vaccines, with females showing not only greater antibody responses to vaccines than males but also more adverse side effects [52]. Because females with Turner's syndrome (45 X0) have only one X chromosome, their immune responses are impaired compared to 46XX females, while males with Klinefelter's syndrome (47XXY) have one X chromosome inactivated and they have a feminized immune response [43, 50].

Since the androgen receptor is also coded for by genes on the X chromosome, sex differences due to the actions of testosterone are regulated by genes on the X chromosome [43]. Since testosterone inhibits immune system activity by upregulating inflammatory cytokines, such as IL-10, and estrogen enhances immune system responses by upregulating pro-inflammatory cytokines, such as TNFa, the genes on the X chromosome modulate sex differences in susceptibility to infectious diseases [43, 50]. While some of the sex differences in disease susceptibility are only evident after puberty, sex differences in immune responses also occur in infancy and old age, suggesting that genes on the sex chromosomes and the sex hormones may have independent effects on sex differences in immune responses [43, 50, 51, 53].

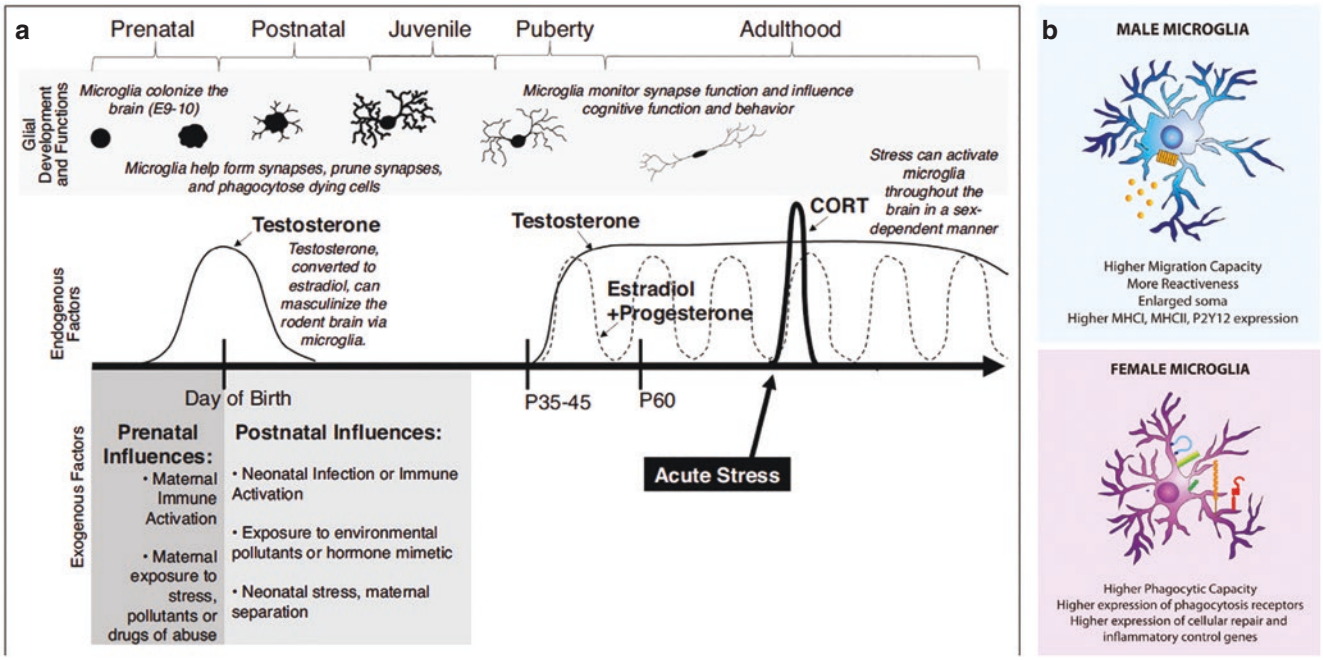

Fig. 11.1 Microglia, hormones, and sexual differentiation during critical periods in the developing brain. (**a**) Microglia and the development of sex differences in the brain. Microglia regulate synapse development and function in the brain, and the development of microglia is regulated by the sex hormones and by environmental stimuli, such as infections, pollutants, endocrine-disrupting chemicals, and stress. Microglia colonize the brain early in development (embryonic day (E) 9–10 in rodents) and regulate the development and function of synapses by initiating synapse formation, pruning aberrant synapses, and phagocytosing naturally dying cells. During the perinatal critical period testosterone masculinizes microglia in males, resulting is male and female microglia. Sexually differentiated microglia influence many neurodevelopmental processes. There are also many perinatal events that can program the function of microglia and later life behavior in a sex-dependent manner. In general, males are more vulnerable to early life insults, including immune activation or stress. Later in life, microglia continue to have an important role in monitoring synapse function and formation, and thereby influencing cognitive function and behavior. During this time, microglia can be influenced by circulating sex steroid hormones, either testosterone in males or estradiol and progesterone in females. Acute stress can also induce the activation of microglia in the brain via glucocorticoid secretion (CORT), in a sex-dependent manner. [**From:** Osborne BF, Turano A, Schwarz. 2018. Sex differences in the neuroimmune system. Current Opinion in Behavioural Sciences. 23: 118-123. Figure 1]. (**b**) Physiological sex differences in male and female microglia. Male microglia have an enlarged soma and more reactiveness in physiological conditions than female microglia. Male microglia also have more pro-inflammatory responses, higher migration capacity, and enhanced Major Histocompatibility Complex, type 1 and 2 (MHCI & MHCII), and adenosine diphosphate receptor P2Y12 gene expression compared to female microglia. Female microglia have a higher phagocytic capacity and higher gene expression of cell repair and inflammatory control genes than male microglia. [**From:** Yanguas-Casas N. 2020. Physiological sex differences in microglia and their relevance in neurological disorders. Neuroimmunology and Neuroinflammation 7: 13–22. Figure 1 [290]]

Sexual Differentiation of the Brain

As discussed in Chap. 1, sexual differentiation occurs during three critical (sensitive) periods (Fig. 11.1): (1) a prenatal critical period during which androgens masculinize males; (2) a postnatal sensitive during which estrogens feminize females; and (3) a pubertal sensitive period during which secondary sex characteristics develop and sex differences in the brain and neuroendocrine system become activated [15, 54–59]. Sexual differentiation during these critical periods involves interactions between genes, sex hormones, the neuroimmune system, and environmental stimuli (Fig. 11.1). At puberty, the surge in gonadal hormones activates the cells that were sexually differentiated during the pre- and postnatal organizational periods. However, puberty is also a critical organizational period for sexual differentiation [60, 61]. The sexual differentiation of the brain during these critical periods determines the organization of sensory, motor, cognitive, and socio-sexual behavior throughout the lifespan [62–65].

There are androgen and estrogen receptors in the brain, each with different spatial distributions (Fig. 11.2). During the critical period of sexual differentiation, testosterone is aromatized to estradiol and binds to E2 receptors to masculinize the brain [55, 66, 67]. The aromatize enzyme is located primarily in those areas of the brain which regulate reproductive behaviors: the hypothalamus and amygdala; however, there are neurons containing aromatase in the hippocampus, cerebral cortex, cerebellum, and spinal cord. Aromatase has also been detected in radial glial cells and astrocytes [68]. Aromatase levels in the preoptic area and

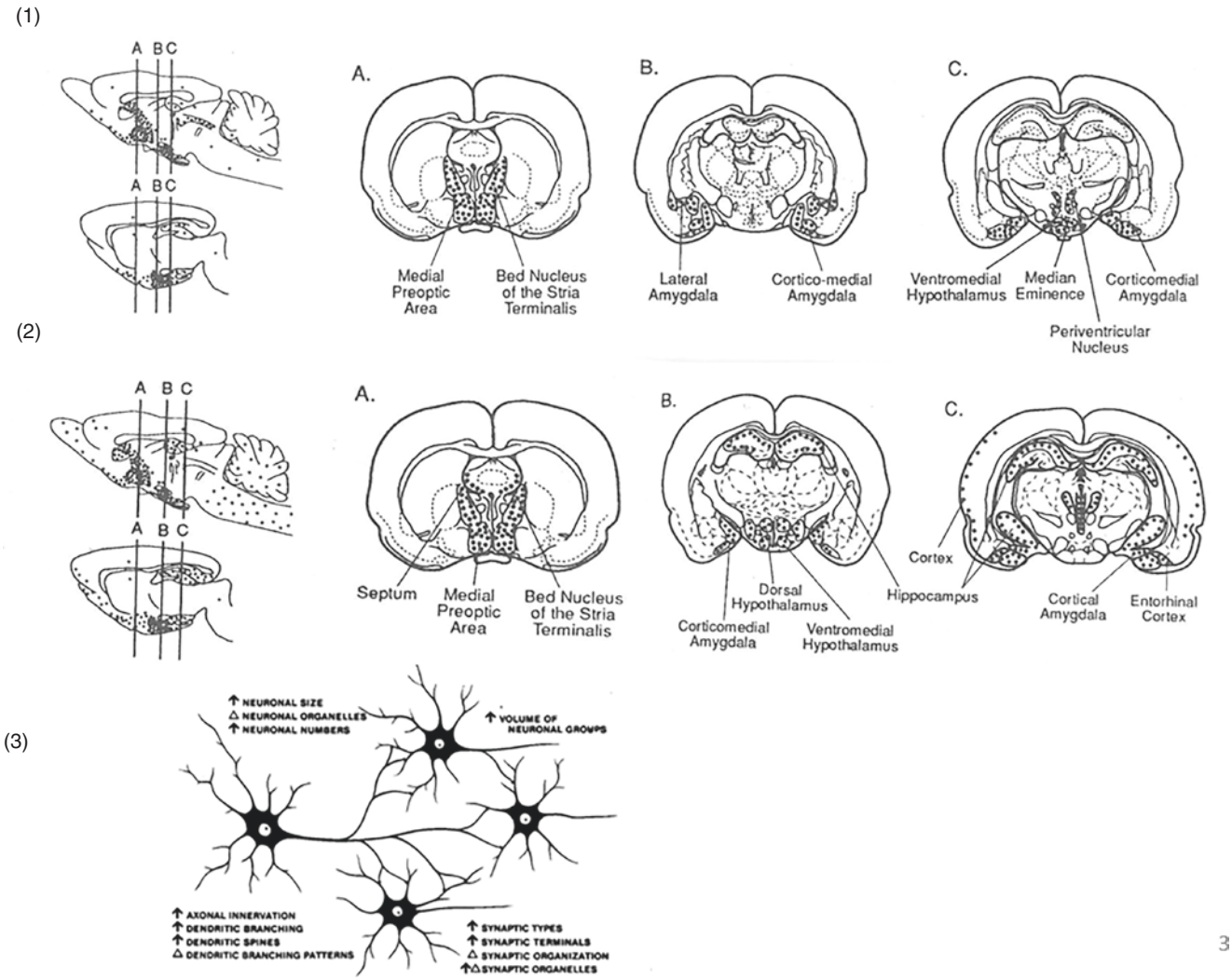

Fig. 11.2 The distribution of estrogen and androgen receptors in the rodent brain. Steroid hormone receptors in the brain. Three coronal (frontal) sections showing the locations of (1) radioactive testosterone and (2) radioactive estrogen uptake in the mouse brain. The lines (A, B, and C) indicate where the sections were cut with respect to testosterone and estrogen receptors shown in sagittal section. [**From**: Brown 1994. An Introduction to Neuroendocrinology. Cambridge University Press, Figures 9.4 and 9.5, [362]]. (3) A drawing of a neural circuit to indicate the ways that steroid hormones can result in sexual dimorphism in the CNS. Neural responses to steroid hormones can result in differences in the growth and development of target axons and dendrites and in the organization and stability of their synapses, thus enhancing neuronal survival and resulting in sex-specific neural circuits with sexually dimorphic neural connections. [**From:** Toran-Allerand CD. 1984. On the genesis of sexual differentiation of the Central Nervous System: Morphogenetic consequences of steroidal exposure and possible role of a-fetoprotein. Progress in Brain Research, 61: 63–98. Figure 5.]

hypothalamus peak during the critical period of sexual differentiation by testosterone, but the timing of aromatase mRNA expression varies in different brain areas, suggesting a range of functions [67, 68].

The aromatization theory indicates that testosterone is aromatized to estrogen which masculinizes the male brain. But the female ovary produces estrogens, so why do not these estrogens masculinize the female? The answer is that alpha-fetoprotein (AFP), which is produced by the fetal liver, binds to circulating estrogens and prevents them from masculinizing and defeminizing females [66, 69]. Excessive estrogens during the prenatal critical period can masculinize the female fetus, producing a number of disorders of female sexual differentiation [55, 70]. While estrogen masculinizes the brains of rodents, androgens binding to androgen receptors appear to masculinize the brains of humans and other primates [71]. However, this is a controversial issue as there is a debate whether it is androgen or estrogen that masculinizes the brains of primates [72, 73]. AFP does, however, have a number of functions during development, including the

regulation of growth, apoptosis, and regulation of the immune system [70]. While estrogens during the prenatal critical period of sexual differentiation will masculinize female rodents, there is a second postnatal critical period (Fig. 11.1), in which estrogens are necessary for feminization [55]. This critical period for estrogens to feminize female rodents appears to be from 15 to 25 days postnatal age. During the early postnatal period (PND 1–15) it appears that estrogens continue to masculinize the female brain but after day 15, they feminize the female brain [74].

Genetic and Epigenetic Factors in Brain Sexual Differentiation

Sex differences in the brain may be due to the expression of the *SRY* gene in the cortex, midbrain, hypothalamus, and SN [75]. Since *SRY* acts as a transcriptional activator and as an activator of epigenetic processes, such as DNA methylation, histone acetylation or methylation, and posttranscriptional regulation of noncoding RNA (ncRNA) or microRNA (mRNA), it can modulate the sexual differentiation of neurons and glial cells [22]. *SRY* gene expression can also modulate the actions of the enzymes TH and monoamine oxidase A (MAO-A) in catecholamine and dopaminergic pathways in the midbrain, resulting in sex differences in these neurotransmitter systems [75]. Sex differences in brain development are modulated by DNA methylation [76]. Masculinization of the brain requires androgens to suppress DNA methylation, while brain feminization is promoted by DNA methylation. Numerous environmental factors, including poor nutrition, drugs, and physical and mental stressors, can alter the epigenetic gene regulation of neural development, resulting in a number of different NDDs [77]. However, unlike gene mutations, which are permanent, epigenetic mechanisms are reversible; thus, NDDs that are caused by epigenetic mechanisms could also be "repaired" by epigenetic reversibility of the environmental stimuli that caused them in the first place: "epigenetic therapeutics": drugs, behavior therapy nutrition, or stress reduction ([77–79]).

Genes, Hormones, and Microglia Interact in the Sexual Differentiation of the Brain

Although the sexual differentiation of the brain is testosterone dependent [80], the sex chromosomes, transcription factors, and microglia are all involved in the sexual differentiation of the brain [16, 19]. Based on the four core genotypes model in which the effects of an XX versus XY genotype without gonadal hormones can be tested, it was found that genes on the Y chromosome coded for an increase in the number of dopaminergic (TH positive) cells in the mesencephalon on embryonic mice [81]. Gonadal steroid hormones activate their receptors in specific brain areas by binding to nuclear transcription factors to active gene transcription and protein synthesis, and the development of sexually dimorphic neural pathways (Fig. 11.2) is modulated by microglia as well as other chemical signals during critical periods [82]. It is hypothesized that microglial cells are sexually differentiated by gonadal steroids during the embryonic organizational period and that they are then involved in modulating the sexual differentiation of particular brain areas [83]. Microglia clear debris from the brain and regulate synaptic communication in adults, but in the developing brain they may eliminate redundant or apoptopic (dead) neurons, modulate synaptogenesis, and regulate the development of neural circuits (See Chap. 1).

Sexual Differentiation of Glial Cells: Astrocytes and Microglia

As noted in Chap. 1, glial cells play an important role in brain development and are intricately involved with synapses, forming a "Tripartite synapse" with the pre-and postsynaptic neurons [84, 85, 86]. Glial cells show sex differences in adults and this differentiation occurs during critical periods of development. However, glial cells also regulate the sexual differentiation of neurons; thus, glial cells are both the targets and promoters of sexual differentiation in the brain [87, 88]. Astrocytes and microglia (Fig. 11.1) are sexually differentiated [89, 90]. Microglia show sex differences in cell number, morphology, and function, which develop during critical organizational periods [83]. There are also sex differences in the number of astrocytes in the medial amygdala in mice and rats [91].

There are three stages of microglial cell development in the brain of rats: the early embryonic period (E10.5–14), the perinatal period (E14–P9), and adolescence (P28 and later) [92]. Humans have two embryonic phases of microglia development; one at four–five weeks into gestation and a second at 10–13 weeks of gestation [92]. There are age-related sex differences in microglia in the amygdala, hippocampus, and nucleus accumbens (NAc) in rats [92] (Fig. 11.1) and microglia are differentially expressed in the cerebral cortex, hippocampus, striatum, and cerebellum of male and female mice [92]. During the embryonic critical period for masculinization of the mPOA, males have twice as many microglia as females and a more activated morphological profile. During the first few days after birth in rats, males have more microglia than females in the sexually dimorphic medial preoptic area of the hypothalamus as well as the amygdala and hippocampus [90]. On the other hand, females have more activated microglia as juveniles and adults (P30–60). The sex differences in the development of microglia at

different ages suggests that during the organizational period there might develop sex differences in the innate immune system which could result in lifelong sex differences in disease susceptibility [92].

There are three mechanisms through which microglia can be sexually differentiated: sex chromosomes, gonadal steroid hormones, and neural environment. Since all glial cells have male or female sex chromosomes, direct expression of the genes on these chromosomes may lead to sexual differentiation of the glial cells. Testosterone may masculinize glial cells during the prenatal critical period by aromatization to estrogen and sex differences in microglia are responsive to the neural environment in which they function [87, 92]. The sex differences in microglia differ in different brain regions; thus, there is not a unitary sex difference: The local activity of growth factors, cytokines, NT, NP, and hormones shapes the maturation of the microglia in each brain area. Sex differences in microglia formed during the prenatal organizational period may persist for a lifetime due to epigenetic programming. Histone acetylation or methylation or DNA methylation may be "imprinted" during the organizational period so that microglia retain the male or female phenotype for life [87] (Fig. 11.1). Finally, environmental stimuli, such as high fat diets, pollutants, stress, infection, or environmental toxins, may disrupt the sexual differentiation of glia and/or disrupt the role of microglia in sexual differentiation [87, 93].

Sex Differences in the Brain

Since every brain cell in males has an X and a Y chromosome and every brain cell in females has two X chromosomes, every brain cell shows a genetic sex difference, and since many brain cells have androgen and/or estrogen receptors, many neural circuits are sexually differentiated [94]. There are many types of sex differences in the brain. Some neurons only develop in one sex, while others are larger in one sex. Sex differences in the brain can be examined in terms of (1) neuroanatomy, (2) neural circuits, (3) neurochemistry, and (4) neuroendocrinology. This has led to the "**mosaic hypothesis**" which proposes that some brain areas are masculinized, while others are feminized [95]. Another way of looking at sex differences in the brain is to distinguish morphological differences in neuron size, shape, or number (**sexual dimorphism**) from biochemical, physiological or pharmacological sex differences in cell function (**sexual diergism**) as defined by Rhodes and Rubin [96]. It has been proposed that neuroinflammatory signals are the "primary drivers of the masculinization of specific brain regions" while steroid hormones modulate the neuroimmune signaling [16, 82]. Sexual dimorphism in neural and glial cells gives rise to neuromorphological cell phenotypes, while sexual diergism in neurochemistry and neurophysiology gives rise to neurochemical phenotypes (see [62]). Both sexual dimorphism and sexual diergism result in sex differences in cognition, emotion, and behavior.

Neuroanatomy

In the human brain, there are sex differences in total brain morphology, with males having larger volumes than females, as well as regional sex differences in gray matter volume and density [97]. Males also have larger amygdala and thalamus volumes than females as well as larger NAc, putamen, and hippocampus [98, 99]. There are sex differences in the size or volume of brain cells in the cortex and cerebellum of humans, as well as in subcortical structures: the thalamus, amygdala, hippocampus, hypothalamus, and many other areas (see [97–101]). However, the distributions of measures of brain parameters for males and females have significant overlap (Joel et al 2019; [99]). There are very few nuclei which have very large sex differences. These include the bed nucleus of the stria terminalis (BNST), the interstitial nucleus of the anterior hypothalamus (INAH1), which is also called the sexually dimorphic nucleus of the preoptic area, the INAH3, and the infundibular nucleus (Joel et al., 2019; [100]). The use of a deep learning technique found sex differences in whole brain measures as well as a number of specific brain areas in the cortex, thalamus, cerebellum, and limbic system of men and women [102]. However, some sex differences in neuroanatomy, particularly in the limbic system, may be influenced by social-emotional factors during development and thus environmental epigenetic stimuli may facilitate brain plasticity during development, resulting in significant individual differences in neuroanatomy within and between sexes [103]. Likewise, there are sex differences in the neuroanatomy in many areas of the rodent brain, including the cortex, hippocampus, olfactory bulb, amygdala, septum, thalamus, and hypothalamus [101, 104, 105].

Neural Circuits

As well as the size of particular brain regions, there are sex differences in the extent of dendritic arborization, the density and pattern of synaptic connections, the size, number, and phenotype of neurons in a particular region and glial cell morphology. All of these structural differences are believed to underlie adult sex differences in behavior. It is not possible to discuss all of the sexually dimorphic and sexually diergic areas of the brain, so I will focus on some of the sex differences in (1) the olfactory pathways, (2) the hypothalamus, (3) the hippocampus, (4) amygdala, and the (5) locus coeruleus (Fig. 11.4) [106–110].

(1) The neurons of the olfactory bulb and the olfactory connections to the medial amygdala, BNST, mPOA, AVPV, and VMH which regulate socio-sexual behavior of mice are sexually differentiated by androgens during the prenatal organizational period (Fig. 11.3) [106–110].
(2) Several hypothalamic nuclei show sexual differentiation during embryonic and pubertal development [100, 105] (Fig. 11.3). These include (1) the sexually dimorphic nucleus (SDN) of the medial preoptic area (mPOA) which is larger in males than females [111–113]. (2) The central region of the BNST is masculinized by androgens and is larger in males [114, 115], while the anterior BNST (the oval nucleus of the BNST) and the ventral BNST (vBNST) are feminized by estrogens and are larger in females than males [116, 117]. (3) The anteroventral periventricular nucleus of the preoptic region (AVPV) of the hypothalamus is larger and contains more neurons in females than in males [111, 118, 119]. (4) There are numerous pathways in the ventromedial nucleus of the hypothalamus (VMH), which are sexually dimorphic regulate sex differences in metabolism and energy expenditure as well as sexual behavior and aggression [120–122].
(3) A number of nuclei in the hippocampus are sexually differentiated by androgens during the prenatal critical period [123], and there are sex differences in synapse formation and dendritic arborization of hippocampal neurons. Males show more CA1 pyramidal neuron dendritic arborizations than females, while females show more primary dendrites in the CA3 area [124, 125]. As in the hypothalamus, the sex differences in the CA1 area may be localized into male-specific and female-

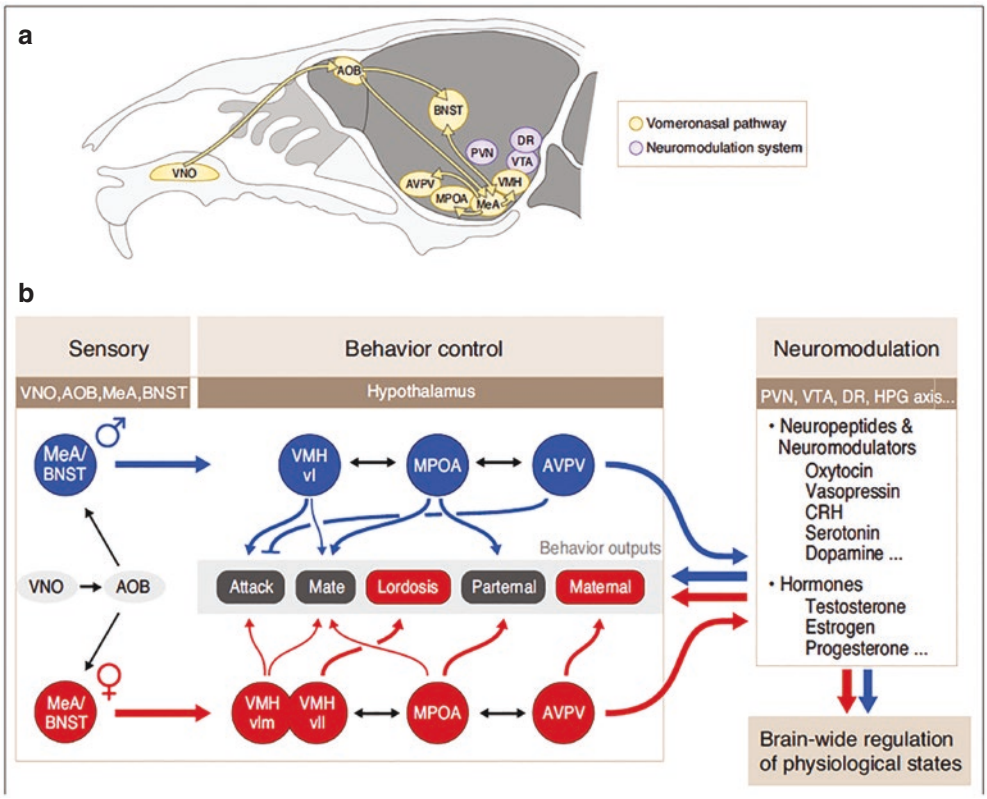

Fig. 11.3 Sex-specific neural pathways in mouse brain. Sexually dimorphic neural circuits involved in the processing of sex-specific social cues in mice. (**a**) Schematic representation of brain regions involved in the processing of sex-specific social cues and in the regulation of social behaviors. (**b**) Control of social behaviors in male (blue) and female (red) mice. Social behaviors, such as aggression, sexual behavior, and parental care, are controlled by different hypothalamic nuclei. Some of these nuclei, such as the ventrolateral part of the ventral medial hypothalamus (VMHvl) and the medial preoptic area (MPOA), drive similar social behaviors in males and females. Others, such as the female lateral part of the VMHvl (VMHvll) and the anteroventral periventricular nucleus (AVPV), are involved in eliciting different behavioral responses in both sexes. Specific hypothalamic nuclei, such as the medial amygdala (MeA) and bed nucleus of the stria terminalis (BNST), receive inputs from the vomeronasal pathway and are regulated by different neuromodulators (hormones and neuropeptides) in a sex-specific manner. *AOB* accessory olfactory bulb, *DR* dorsal raphe nucleus, *PVN* paraventricular nucleus, *VMHvl* ventrolateral part of the ventral medial hypothalamus, *VMHvll* lateral part of the VMHvl, *VMHvlm* medial part of the VMHvl, *VNO* vomeronasal organ, *VTA* ventral tegmental area. [**From:** Li Y and Dulac C. 2018. Neural coding of sex-specific social information in the mouse brain. Current Opinion in Neurobiology. 53: 120–130. Figure 1 [363]]

specific pathways, but these differences may be labile and depend on the activational effects of hormones at puberty and on epigenetic activation of gene expression in particular neurons [126, 127]. Bundy et al. [128] found over 60 genes that were differentially expressed in the hippocampus of male and female mice, suggesting that many sex differences have yet been described. The hippocampus functions in cognitive and emotional behavior [129, 130] and sex differences in cognitive function, depression, and NDDs involve the hippocampus [125, 127, 131].

(4) The medial amygdala is responsive to changes in androgen levels in adulthood and shows morphological plasticity with circadian, annual, and socially modulated androgen levels [132]. Sex differences have been shown in the anatomy of the amygdala [97, 133, 134], with some nuclei being masculinized and some feminized. The medial amygdala is intricately associated with olfactory pathways (see Fig. 11.3) and olfactory stimuli from conspecifics (Pheromones) activate different sexually dimorphic neurons in the medial amygdala in males and females [106, 135].

(5) The Locus coeruleus of females has a larger volume and a greater number of neurons than in males and it appears that the number of such cells is reduced by apoptosis postnatally in males, while estrogen modulates the survival of these neurons in females [136–138]. The locus coeruleus (LC) of females has more noradrenergic neurons than males and these have larger and more complex dendrites than those of the male LC [136].

Neurochemistry

As noted by Nieuwenhuys [139], the chemical signaling pathways of the brain transect its neuroanatomical regions. Many neurons have steroid hormone receptors and these steroid hormone target neurons are susceptible to sexual differentiation (Figs. 11.2). Thus, although the neuroanatomical regions of the brain, such as the medial preoptic area, ventral hypothalamus, hippocampus, and amygdala, can be defined morphologically, these anatomical regions may contain a plethora of neurochemically differentiated neurons which use different neurochemicals and have been sexually differentiated by androgens or estrogens during critical periods [122, 140–142]. There are sex differences in a number of neurochemical systems in the brain. These examples of ***sexual diergism*** mean that there can be functional differences in chemical signaling and intracellular communication that are not measurable in terms of brain morphology [96]. These include the locus coeruleus noradrenergic system [136]; the cholinergic system [143, 144]; and sex differences in GABA release in the neurons of the BNST [145, 146]. There are also sex differences in neuropeptide signaling systems, including enkephalin, CCK, and beta endorphin (See [147]).

There are many different patterns of sex differences in neural signaling pathways.

(1) The neurons in one sex can be eliminated by apoptosis, while those in the other sex are rescued by neurohormone actions. Such apoptotic processes may involve steroid hormone activation of glial cells, immune cells, and epigenetic activation of genes regulating either cell death or cell survival pathways. (2) There is a larger number of neurons in a particular neuroanatomical area in one sex than in the other. (3) There are more dendritic spines and/or synapses on neurons in one sex than in the other. (4) There are sex differences in the pattern of neurochemical signals in the neurons of each sex. (5) There is differential activation of intracellular biochemical pathways in each sex resulting in differences in gene expression. (6) There are sex differences in epigenetic responses of neurons to the same neurochemical stimulation resulting in sex differences in gene expression. (7) The responses of neurons to sex hormones is plastic, depending on the environment so that the same neuron may under some circumstances express a female pattern and under other circumstances express a male pattern. (8) There are sex differences in the actions of glial cells. (9) There are sex differences in the actions of immune-related cells and the release of cytokines. The end result is that a cascade of neurochemical signals results in a sexually dimorphic **pattern of gene expression** in a cell which regulates sex differences in physiology, metabolism, and behavior (Fig. 11.4) [140, 142, 148].

Summary: Sex Differences in Brain Structure, Function, and Neurochemical Pathways

The study of sex differences in the brain and their relationship to NDDs is undergoing a paradigm shift based on the availability of more and more sensitive molecular and genetic technologies. Whereas the initial studies focused on morphological differences in particular neuroanatomical regions, the newer studies focus on gene expression and molecular differences in specific neurons. For example, Labonte et al. [149] identified sex-specific gene expression patterns in six brain regions associated with sex differences in major depressive disorders (MDD) in humans [the ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex, dorsolateral PFC, anterior insula (aINS), NAc, and ventral subiculum (vSUB)]. This seems to be the future of studies on sex differences in the brain underlying neurodevelopmental and neurodegenerative disorders. The key breakthrough, however, will involve understanding how sex differences in gene expression and molecular biology lead to sex differences in neurochemical communication and synaptic function, because synaptic damage seems to underlie all neurodevelopmental

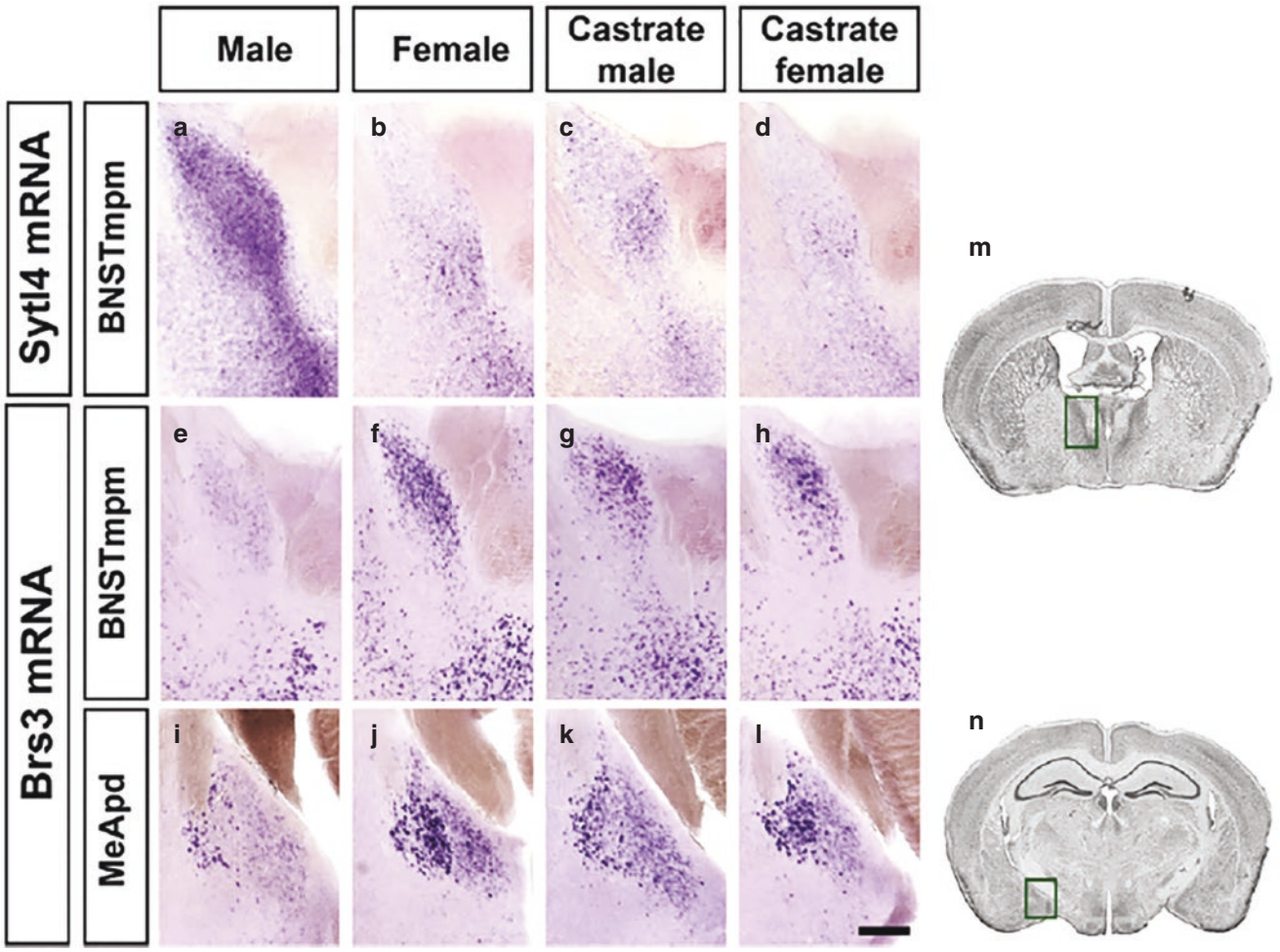

Fig. 11.4 Sex differences in gene expression patterns in the brain. Sex differences in gene expression patterns in mice are regulated by gonadal hormones. Sexually dimorphic mRNA expression of Synaptotagmin Like 4 (**Sytl4**) and Bombesin Receptor Subtype 3 (**Brs3**) genes in coronal sections from the brains of male and female, castrated male and castrated (ovariectomized) female mice. There is more Sytl4 mRNA in the posteromedial area of the medial bed nucleus of the stria terminalis (BNSTmpm) in males than females (**a**–**d**), but there is more Brs3 mRNA in the BNSTmpm (**e**–**h**) and posterodorsal Medial Amygdala (MeApd) (**i**–**h**) of female mice than males. The boxed areas of the Nissl stained brain sections (M and N) show the BNST (**m**) and MeA (**n**) regions shown in sections **a**–**h** and **i**–**l**, respectively. The scale bar (**a**–**l**) represents 100 mm. [**From:** Xu X, Coats JK, Yang CF, Wang A, Ahmed OM, Alvarado M, Izumi T, Shah NM. 2012. Modular genetic control of sexually dimorphic behaviours. Cell. 148: 596–607. Figure 2 [142]]

and neurodegenerative disorders [150–152]. Synaptic organization underlies neural development during critical periods [153] and both pre- and postsynaptic molecules are involved in NDDs [152, 154–157]. Sex differences have been found in almost all neurochemical systems, including those underlying neurodevelopmental and neurodegenerative disorders and sex differences in these neurotransmitter and neuropeptide pathways occur throughout the brain [147, 158, 159]. Sex differences at synapses (Fig. 11.2) can occur in the release of neurotransmitters from pre-synaptic nerve terminals or in their receptors at pre- and postsynaptic nerve terminals [160], or in the excitatory/inhibitory synapse ratio [161, 162]. As noted below, sex differences may also occur in the contribution of glial cells to "tripartite synapses," the neuro-immune system or even the communications between the gut microbiome and the brain [163].

The Role of Glial Cells in the Sexual Differentiation of the Brain

Astrocytes are involved in synapse development and function and microglia regulate neuron cell number during development and facilitate cell proliferation and differentiation and may play a role in the sexual differentiation of brain areas by regulating apoptosis (See Chap. 1). Microglia also regulate axon outgrowth and synaptogenesis; modulating activity-dependent synapse formation by eliminat-

ing weak synapses and promoting the growth of new ones [88]. Microglia play different roles at different times during development, but at each developmental period, they are involved in the sexual differentiation of the nervous system (Fig. 11.5). During embryonic development, microglia have organizational effects through the promotion of neural proliferation and cell survival by secreting cytokines which facilitate neural development and neural circuit development and abnormalities during this period have lifelong effects. During this embryonic critical period, testosterone masculinizes the POA by stimulating microglia to facilitate the development of synaptic spines [164]. In females, microglia appear to function postnatally to feminize the brain by facilitating synapse development during the first few postnatal weeks [165]. Later microglia prune superfluous cells by inducing apoptosis and facilitate axonal

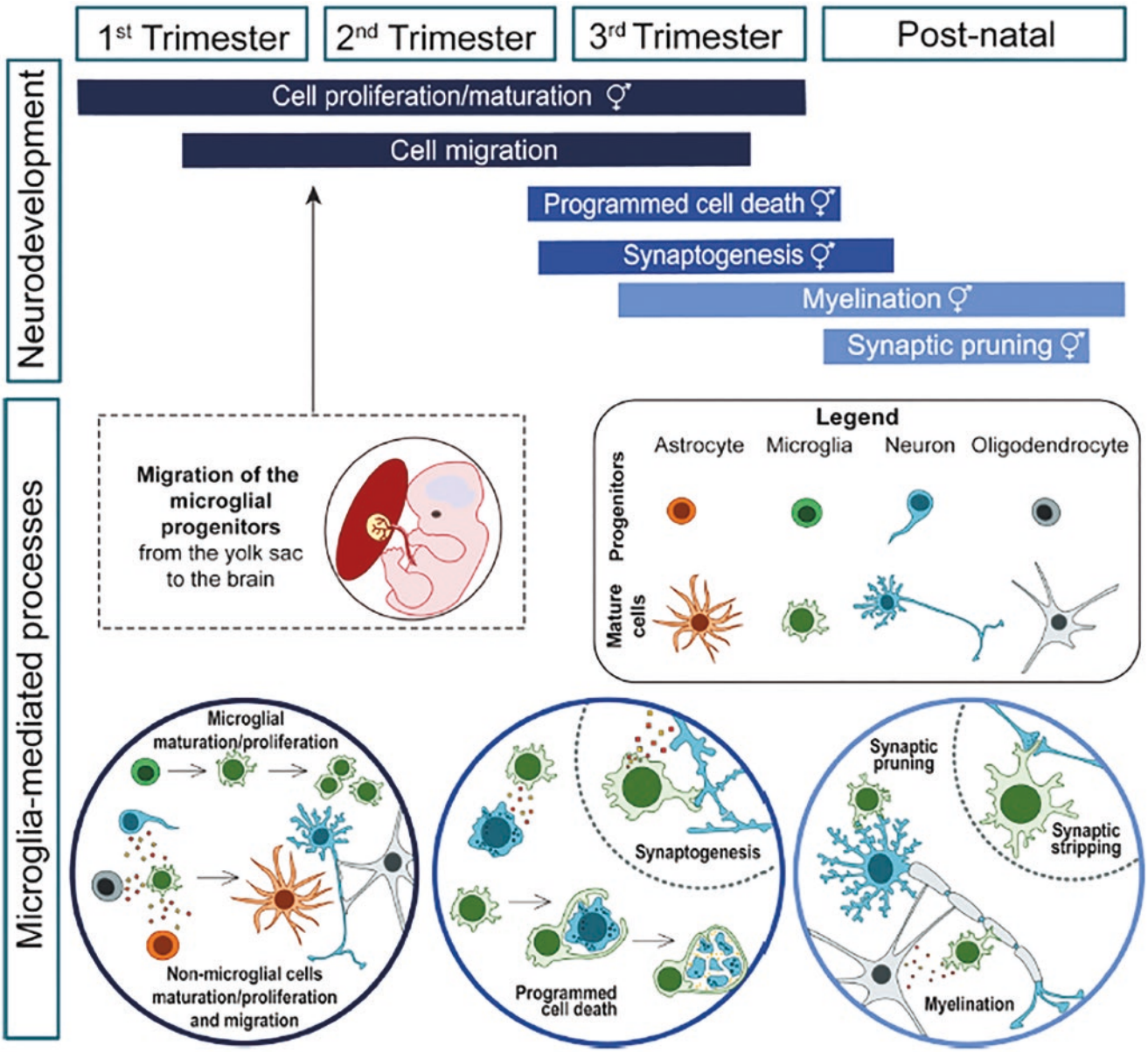

Fig. 11.5 Microglia and sexual differentiation of the brain. A timeline showing the influence of microglia on neural development. After microglial progenitors migrate into the brain from the embryonic yolk sac, microglia proliferate and participate in the neurodevelopmental processes involving astrocytes, neurons, and oligodendrocytes, including programmed cell death, synaptogenesis, myelination, synaptic pruning, and synaptic stripping. Astrocytes, microglia, neurons, and oligodendrocytes are, respectively, represented in orange, green, teal, and gray, where the microglial progenitors possess a rounded shape compared to mature cells. Sexually dimorphic processes of neurodevelopment are indicated by the symbol o. [From: Bordeleau M, Carrier M, Luheshi GN, Tremblay M-E. 2019. Microglia along sex lines: From brain colonization, maturation and function, to implication in neurodevelopmental disorders. Seminars in Cell & Developmental Biology. 94: 152–163. Figure 1 [169]]

outgrowth and synaptic connectivity by promoting synaptogenesis and pruning excess synapses [87]. In adulthood, microglia facilitate experience-dependent synaptic remodeling by regulating the interface between environmental (epigenetic) stimuli and synaptic changes in neural circuits [88]. These effects are more like activational effects, but they are susceptible to disruption by environmental stressors or endocrine disruptors.

The Role of Glial Cells in Sex Differences in NDD

Given the complex interactions between astrocytes, microglia, and neurons in the sexual differentiation of the brain, it is no surprise that glial cells may be involved in the sex differences in NDDs [166, 167]. Astrocytes are essential for synapse formation and synaptic function and sex differences in gliotransmission may be involved in the sex differences found in NDD [84, 156]. Thus, the role of astrocytes in sex differences in NDD may be in their role in the tripartite synapse and the modulation of synaptic activity, possibly by regulating the E/I ration of synapses [168]. Neuroinflammation is an important component of a number of neurodevelopmental and neurodegenerative diseases and microglia may mediate sex differences in NDD by regulating neuroimmune responses during early development as well as in adulthood and aging [11, 169]. This could occur through sex differences in the elimination of synapses by microglia during synaptic pruning, or through sex differences in the neuroimmune responses of microglia to external (environmental) stimuli (Fig. 11.5). Since microglia are involved in sexual differentiation, they may be involved in X-linked NDDs. Finally, the sex hormones, estrogen and testosterone, may have differential effects on microglia [170], being neuroprotective or neurotoxic, depending on the neural environment. For example, chronic stress has differential effects on astrocytes and microglia in males and females and this difference may be mediated by the sex hormones [171]. Likewise, during a state of low oxidative stress, sex hormones may be neuroprotective, but during high oxidative stress they may be neurotoxic [172]. Nevertheless, it is becoming increasingly clear that microglia are involved in the sexual differentiation of the brain, in sex differences in neuroimmune activation and in the development of sex differences in neural dysfunction in infancy, adulthood, and aging [169, 173].

Gut Microflora, the Immune System, Glial Cells, and Sex Differences in NDD

As if things were not too complex already, the role of gut bacteria, the microbiome, adds yet another factor in the causal chain of sex differences in NDD. The microbiome and microglia communicate with one another through a number of chemical signals, including neurotransmitters (serotonin, noradrenaline, or dopamine), short-chain fatty acids (such as propionic acid (PPA), acetic acid (AA), and butyric acid (BA)), cytokines, and microbial-associated molecules (such as bacterial lipoproteins, double stranded RNA and lipopolysaccharides) [174]. Secondly, the microbiome may act as an epigenetic regulator of microglial phenotypes, thus influence microglial function during development [175]. Thirdly, through their communication with the microglia and other immune functions, the microbiome can influence the development of NDD [176]. Finally, there are sex differences in the gut–brain axis [177], which can contribute to the sex differences in the onset of NDD [178]. A microbial imbalance in the gastrointestinal tract (gut dysbiosis) seems to be one of the major causes of sex differences in NDD. It has been proposed that sex differences in gut microflora may underlie sex differences in metabolic disorders [179]; major depressive disorder [180]; autism spectrum disorder [178]; and other neuropsychiatric disorders (see [181]). The microbiome–immune–microglia interactions can be activated by environmental stressors (early life adversity) and affect the functioning of the autonomic nervous system in a sex-dependent manner [182], resulting in sex differences in stress-related behavior and sympathetic nervous system activation, which underlies neurovascular disorders, such as hypertension [183]. The gut microbiome may modulate sex differences in neural development through communication with the immune system via microglia [169, 174, 184] and led to sex differences in neural development and in a wide range of NDD and other neural disorders [177]. Figure 11.6 presents an overview of the complex interactions between the gut microflora, immune system, neuroendocrine system, genes, and environmental stimulation in the sexual differentiation of the CNS.

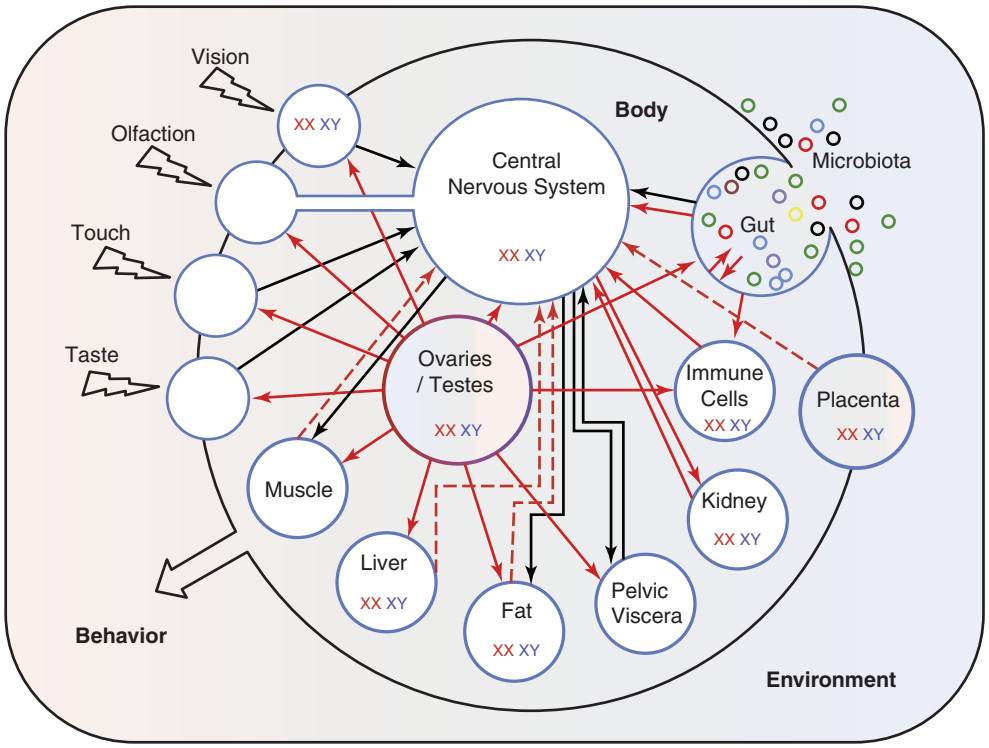

Fig. 11.6 Complex interactions in the sexual differentiation of the CNS. A diagram summarizing the factors influencing sex differences in the central nervous system. The central nervous system is embedded in a sexually differentiated body. Solid arrows indicate a sex influence from one organ on another. Dashed arrows indicate an influence inferred from circumstantial evidence. Black arrows indicate neural communications; red arrows indicate humoral communication. "XX XY" indicates organs in which sex chromosome complement has a demonstrated effect, either directly within that organ or indirectly via effects on other organs. The body is embedded in an environment that affects the individual in a sex-dependent manner. The small colored circles in the upper right are the many species of microorganisms (microbiota) living commensally in our gut or on our skin. On the left, vision, olfaction, touch, and taste may all be processed differently in males and females. [From: De Vries, G.J., Forger, N.G., 2015. Sex differences in the brain: a whole body perspective. Biol. Sex Differ. 6, 15. Figure 1 [364].]

Sexual Differentiation of the Hypothalamic-Pituitary (Neuroendocrine) System

The pituitary gland has two distinct parts: the adenohypophysis (anterior pituitary) and the neurohypophysis (posterior pituitary), each of which is connected to the hypothalamus [185, 186, 187]. The posterior pituitary (neurohypophysis) is formed from neural tissue, while the anterior pituitary (adenohypophysis) is a true endocrine gland. During development the two parts of the pituitary gland become intertwined through a complex series of genetic, epigenetic, and hormonal interactions [185, 188–194]. The hypothalamic-pituitary system is sexually differentiated in a number of complex ways. The neurohypophyseal system consists of the neuroendocrine cells of the Supraoptic nucleus (SON) and the Paraventricular nucleus (PVN) of the hypothalamus which synthesize the neurohormones oxytocin and vasopressin, and which send their axons down the hypophyseal stalk (Infundibulum) to the Pars nervosa or posterior pituitary gland where these hormones are released into the bloodstream [195, 186]. In addition to their hormonal function in the bloodstream, oxytocin and vasopressin are also released into neural pathways in the brain where they act as neuromodulators [196–198].

The adenohypophysis (anterior pituitary) contains five types of endocrine cells which secrete growth hormone, prolactin, LH and FSH, ACTH, and TRH as well as cells in the pars intermedia which secrete MSH [186]. Synthesis and secretion of the hormones of the adenohypophysis is regulated by the hypothalamic hypophyseal hormones, which are secreted from the parvocellular neurons of the PVN, ventromedial nucleus, and other nuclei of the hypothalamus and released into the bloodstream in the median eminence of the hypothalamus, from which they travel down the hypophyseal portal veins of the pars tuberalis in the pituitary stalk to the adenohypophysis. As with the neurohypophyseal hormones, the hormones of the adenohypophysis also act as neuromodulators in the brain

Neurohypophyseal Hormones and Sex Differences in NDD

Both vasopressin and oxytocin may mediate sex differences in NDD involving social behavior and anxiety-related behavior. The lateral septum AVP system is involved in sex differences in social recognition, social play, and anxiety-related behavior in rats, while the mPFC-OXY system is involved in socio-sexual motivation in female mice and anxiety-related behavior in mice [199]. It appears that OXY and AVP have some neuromodulatory effects on sex differences in NDD, but the effects are quite complex and their effect may be to shift the excitatory/inhibitory NT balance in subtle ways to influence both vulnerability and resilience to the development of NDD. Oxytocin and vasopressin systems have been implicated in Autism spectrum disorder, Prader-Willi syndrome, Williams syndrome, and Fragile X syndrome; however, the exact role of these neuromodulators in these disorders is unclear [200]. Given the importance of oxytocin in social and sexual behavior, there has been considerable attention to its role in Autism Spectrum Disorder [201]. The amount of oxytocin released, the number of oxytocin receptors, and the function of glycoprotein CD38, which is present on the surface of many immune cells (including $CD4^+$, $CD8^+$, B lymphocytes and natural killer cells) and also functions in cell adhesion, signal transduction, and calcium signaling, have all been implicated in ASD. However, the use of oxytocin analogues to treat ASD has met with little success [202, 203]. On the other hand, the social behavior and oxytocin abnormalities in ASD may be the result of dysfunctions in synaptic proteins, such as Neuroligan3 (NLGN3 gene) [204], a sex-linked gene found in the X chromosome [205]. In addition, genetic polymorphism and epigenetic modulation of oxytocin receptors have been implicated in ASD [206–208]

Adenohypophyseal Hormones, Sex Differences, and NDD

A complex spatio-temporal sequence of neurochemical and genetic events are involved in the sexual differentiation of the neuroendocrine hypothalamus during the fetal organizational period [209, 210]. The GnRH neurons are born in the olfactory placode and must migrate to the preoptic area of the hypothalamus. This is under the control of a number of genes, growth factors, cell adhesion molecules, and other chemical signals. Once the hypothalamic GnRH neurons develop, they begin to secrete GnRH which activates the pituitary and gonads. GnRH release is pulsatile and the "pulse generator" differs in males and females [211]. In males, androgens from the testes provide negative feedback, resulting in small regular pulses of GnRH. In females on the other hand, estrogen provides positive feedback, resulting in surges of GnRH secretion once every reproductive cycle (estrus cycle in rats, menstrual cycle in humans). However, the sex difference in the HPG feedback system is not regulated directly by the GnRH neurons themselves, which are not sexually dimorphic, but indirectly by a complex system of neurotransmitters and neuropeptides [212, 213], of which the kisspeptin neurons are the most important. The kisspeptin neurons are located in the MPOA/AVPV and in the Arcuate nucleus (ARC) and are sexually differentiated by gonadal steroids during prenatal critical periods [214]. Prenatal androgens promote the development of kisspeptin neurons in the ARC but not in the AVPV in males, while postnatal estrogen facilitates kisspeptin neuron development in the AVPV in females [214].

As well as kisspeptin, other neuropeptides and neurotransmitters are involved in the regulation of GnRH secretion. These include the neurotransmitters Glutamate, GABA, dopamine and noradrenaline, and the neuropeptides leptin, NPY, VIP, neurokinin B, and dynorphin as well as GnRI, gonadotropin inhibitory hormone [186, 210, 212, 215]. In mice, AVP and VIP stimulate kisspeptin neurons in the ARC more in females than in males, but Neurokinin B stimulates kisspeptin release equally in males and females [216]. The ability of neurotransmitters and neuropeptides to regulate GnRH secretion means that any epigenetic (environmental) factors that alter these neurochemical signaling pathways can affect the kisspeptin-GnRH release [212], thus providing a neural pathway for endocrine disruptors of many types to influence the hypothalamic-pituitary hormone systems. As if all these were not enough, the glucocorticoid hormones of the HPA system and the thyroid hormones of the HPT system also modulate GnRH release in a sex-specific manner through their effects on Kisspeptin, GnRH, and GnRI [212, 215].

HPG System

Failure of the HPG system to develop normally affects sexual differentiation, puberty, physical development, and reproduction. Mutations of kisspeptin or the KISS1R lead to disorders associated with pubertal development, such as precocious puberty and idiopathic hypogonadotropic hypogonadism. Failure of the GnRH neurons to develop normally can result in hypogonadotropic hypogonadism or in Kallmann syndrome or hypogonadotropic hypogonadism with anosmia (Table 11.2). In this case the GnRH neurons fail to migrate from their origin on the olfactory bulb to the hypothalamus and the olfactory neurons also fail to develop [217]. In this condition, there is no GnRH secretion, so puberty is delayed, and people are infertile. It is treated with gonadal hormone replacement therapy [218]. Males with GnRH dysfunction who have hypogonadotropic hypogonadism have abnormal kisspeptin – Kiss-R1 receptor development [219]. On the other hand, early activation of the

Kisspeptin-GnRh system can result in precocious puberty in both girls (under age 8) and boys (under age 9) [217].

HPA System

The general consensus [220] is that females are more vulnerable to the long-term effects of early life stressors than males. Sex differences in the HPA system are most evident in responses to stress, which is associated with several psychiatric disorders that occur more frequently in women than men, including panic attacks, anxiety disorders, posttraumatic stress disorder (PTSD), and depression [221]. The stress response involves the activation of the HPA system and for some disorders, such as depression, women seem to have a stronger HPA activation than males; however, the evidence is controversial and sex differences in the HPA response to stress may differ between rodent models and humans [222]. Studies in rodent models indicate that females have a greater HPA response to stress than males, with a greater release of CRF, ACTH, and corticosterone [221, 223, 224]; however, in humans the sex difference in the cortisol response to stress depends upon the stressor [225]. After an extensive review of the literature, [220], concluded that there was evidence of increased HPA axis reactivity to stressors in human females compared with males, but this depends on a number of developmental and environmental factors and on the nature of the stressors. The HPA system in human males is activated more by cognitive and verbal stressors, while the HPA system of females is activated more by social stressors [225]. In addition, females have a greater HPA response in depression than males. Thus, it is clear that sex differences in the HPA system and the response to stress are very complicated.

Two results of the increased sensitivity of girls to HPA axis activation are the stress-related inhibition of puberty and the increase in major depressive disorder in females [226]. These sex differences in stress-related disorders may involve sex differences in the functions of the hypothalamic CRH which acts as a neuropeptide through its receptors in the brain, as well as a hormone acting at the adenohypophysis [222, 227, 228]. Sex differences in the HPA response to stress and the **development of emotional disorders** may be the result of early life stressors which affect the "developmental programming" of the HPA axis during critical periods of development [229, 230]. Early life stressors of many types (maternal stress, maternal deprivation, maternal nutrition, social stress, environmental stressors, inflammation) can act as epigenetic factors to shape sex differences in HPA responses to stress [228]. These epigenetic effects can occur through the neural pathways or through the involvement of the immune system and glial cells, such as astrocytes which modulate the development of the HPG neuroendocrine system [231–233]. Early life stress can alter the development of serotonin receptors (Htr2a and Htr1a) in the amygdala and the effects of stress on these receptors differs in male and female rats [234]. Early life stress increases the number of presynaptic Htr1a receptors and decreases the number of postsynaptic Htr2a receptors in females, but has little effect on males, thus indicating an epigenetic mechanism for the sexually dimorphic effects of early life stress on anxiety-related behavior, at least in rodents in this study. Sex differences in the effects of prenatal stress on the HPA system may be the result of sex differences in the responses of DNA methylation to early life stressors. In rats, for example, chronic restraint stress of pregnant females results in sexually dimorphic differences in the HPA axis [235], and in DNA methylation in the GR gene, resulting in sex differences in stress-related behaviors in adulthood [236]. Early life stressors induce sex-specific developmental changes in the neural connections between the PFC, amygdala, and hippocampus in rodents [237] and these connections may mediate sex differences in behavioral and HPA responses to stressors. Such epigenetic changes in neural pathways following early life stressors during development may be the cause of sex differences in major depression and other emotional disorders related to the HPA axis [238].

The HPT System

Congenital hypothyroidism is the most common endocrine disorder in newborn children and is the leading cause of preventable mental retardation [239]. Hypothyroidism (congenital, autoimmune) is sexually dimorphic [240, 241] with three times as many girls affected as boys (3:1 sex ratio) [242]. Females are also more prone than males to develop autoimmune thyroid disorders during puberty [240, 243] and since these disorders are caused by anti-thyroid antibodies produced by immune system, there is a neuro-endocrine-immune interaction in the sex differences in the development of thyroid disorders [244]. There are three other aspects of sex differences in HPT system that are related to NDD disorders: (1) metabolism and the growth spurt during puberty and obesity, (2) the sex difference in depression, and (3) epigenetic effects from environmental pollutants.

The HPT system is essential for thermoregulation and regulates energy balance by controlling energy expenditure, heat production, and metabolism [245]. Hypothyroidism results in lowered metabolic rate and hypothermia as well as delayed physical and mental growth. Since thyroid hormones stimulate bone growth and maturation, hypothyroidism is also associated with short stature. Thyroid hormones play an important role in the pubertal growth spurt and sex differences in body weight [246]. At puberty there is a surge in thyroid gland growth in both males and females, and after menarche, females have larger thyroid glands than males [246]. While GH and IGF1 may drive the increase in thyroid hormone growth at puberty, the sex difference may be the result of estrogen acting on the thyroid gland [246, 247]. Females are diagnosed with thyroid disorders, both hypo- and hyperthyroidism and are more likely to have thyroid autoim-

mune disorders than males [243, 248]. Sex differences in body weight control involve the regulation of energy homeostasis by the HPT system [249] and obesity related to hypothyroidism and autoimmune disorders is more common in women than men (see [250]). Sex differences in body fat distribution are related to sex differences in levels of circulating leptin and other adipokines [251]. Leptin levels rise at puberty and may be responsible for sex differences in fat mass after puberty: Girls gained more fat mass than boys, whereas boys gained more fat-free mass [252]. Leptin and other adipokines stimulate the immune system and since females have higher levels of leptin than males, this may be one underlying cause of sex differences in autoimmunity in general and in thyroid autoimmune disorders in particular [244]. Thus, the sex differences in body composition that occur during puberty reflect differential activity of the HPG system, the HPT system as well as leptin and other adipokines.

In addition to cognitive impairment, hypothyroidism may result in anxiety, depression or bipolar disorder, restlessness, psychomotor retardation, decreased appetite, fatigue, lethargy, and impaired concentration [248]. Women have a higher frequency of mood disorders than men, including unipolar depression and bipolar disorder which may be related to thyroid dysfunction [248]. Among patients hospitalized for depressive disorder and other neuropsychiatric disorders, females are more likely than males to have hypothyroidism [253, 254]. Low levels of TSH during development have also been associated with higher levels of ADHD in girls, but not boys [255].

Environmental toxins which disrupt the development of the HPT system (Thyroid-disrupting chemicals) have widespread effects on brain development [256]. Many of the same chemicals that disrupt the HPT system also disrupt the HPG system and thus may result in sexually dimorphic abnormalities in neural development [256, 257]. There are many endocrine-disrupting chemicals, and Bisphenol A (BPA) can be used as an example of a chemical which disrupts multiple neuroendocrine systems, including the HPT and HPG systems [258]. **BPA** is both an estrogen disruptor and a thyroid hormone disruptor, as it can interact with ER, AR, and thyroid hormone receptors (TRs) as well as other nuclear hormone receptors [259]. BPA inhibits thyroid hormone secretion and blocks TRs while stimulating estrogen receptors. It has been implicated in the increase in ADHD and related behavioral deficits in boys and in a reduction in cognitive function, but it also affects the immune system, insulin, and glucose metabolism and adipose tissue [256, 258, 260, 261]. There are sex-specific effects of prenatal BPA on NDD, suggesting that the interaction of the endocrine-disrupting effects of BPA on the sex hormones and thyroid hormones results in different NDD in boys and girls [262, 263]. Endocrine disruptors, such as BPA alter DNA methylation, by modulating the DNA methyltransferase enzymes (DNMT1 and DNMT3A) in estrogen receptors in the prefrontal cortex and hypothalamus differentially in males and females [264]. By altering DNA methylation and histone acetylation, BPA can cause widespread disruption of reproductive development, particularly in males [265]. Likewise BPA disrupts DNA methylation and histone modifications on androgen receptors and other hormone receptors [266]. Finally, early life anesthesia can result in neurodevelopmental abnormalities which differ between males and females [267]. General anesthetics can cause neural death (apoptosis) and thus act as neurotoxins during early brain development. Since anesthetics target GABA and NMDA receptors, they affect the development of the cortex, hippocampus, and hypothalamus through altering DNA methylation and histone proteins [267]. General anesthetics may also act as endocrine disruptors to alter the development of the HPT and immune systems [268].

GH and Prolactin

Short stature has been associated with a number of NDD, and this may be related to abnormalities in the HPT or GH/IGF1 systems [269]. The GH/IGF1 system has significant effects on brain development and synaptic function and abnormalities in the development of this system may underlie the development of certain NDD, such as Autism and RTT [270–272]. Abnormal synapse formation in Autism may be related to abnormalities in IGF1 levels, in the hippocampus and cerebellum [271]. The fact that the GH/IGF1 system is sexually dimorphic with increased activity in males, suggests that a dysfunction in this system during development may underlie the preponderance of males in autism and related NDD. There have been attempts to correlate elevated prolactin levels with the onset of psychoses in men and women [273], but although women had higher PRL levels than men, the results showed no relationship between prolactin levels and sex differences in psychoses. On the other hand, Labad [274] proposed that sex differences in the HPA system and PRL response to stressors may underlie the onset of schizophrenia.

Summary and a Caveat

Section "Puberty: The Organization of the Adult Brain and the Integration of Sex Differences in the Neuroendocrine, Neuroimmune, and Energy Homeostasis Systems" examines sex differences in the hypothalamic-pituitary hormone systems and suggested ways in which these could underlie sex differences in NDD. However, there is a significant difficulty in the study of sex differences in the neuroendocrine system underlying NDD in that everything depends on everything else, and the causal chain is a circle. For example, the HPG system is sexually dimorphic and sex hormones masculinize

or feminize certain brain regions, leading to sex differences in the hypothalamic-pituitary release of hormones and then these hormones bind to their receptors in the brain, in a sexually dimorphic manner. So what came first: the sexual differentiation of neurons causing sexually different hypothalamic-pituitary hormone release OR the sexually different hormone release causing sex differences in neural development? One might argue that both are true at different developmental periods. During the perinatal organizational phase the gonadal hormones cause sexual differentiation of brain areas and then during the pubertal-adult activational phase, the sexually differentiated brain areas cause sex differences in hormone secretion. However, brain damage – abnormal neurogenesis and synaptogenesis – may cause both NDD and neurohormone abnormalities. The development of the brain and the neuroendocrine system, not to mention the neuroimmune system, are intricately linked and almost impossible to dissociate.

Puberty: The Organization of the Adult Brain and the Integration of Sex Differences in the Neuroendocrine, Neuroimmune, and Energy Homeostasis Systems

Puberty defines the border between childhood and adulthood. It involves the maturation of sex differences in the brain and the initiation of reproductive function. Puberty is both a period of activation of the sexually dimorphic neural pathways that were organized during the perinatal critical period and also a period of the adult organization of the sexually dimorphic neuroendocrine pathways. During puberty there is the maturation of the gonads, the development of secondary sexual characteristics, accelerated growth, changes in brain and behavior, and the attainment of reproductive fertility [61, 275, 276]. Puberty involves sex differences in gene expression underlying the reorganization and activation of many neurochemical systems, including the HPG, HPA, HPT, GH/IGF1, and leptin-related metabolic systems. Puberty also involves the sexual differentiation of the neuroimmune system. Because puberty is a critical period for the development of adult sex differences, the neuroendocrine changes during this time are susceptible to alteration by endocrine disruptors and other epigenetic mechanisms that can result in puberty-related NDD, including disorders of metabolism; early (precocious) of delayed puberty; disorders of sexual differentiation; psychiatric disorders; and disorders of gender identity and role.

Puberty-Related Neural and Neuroendocrine Reorganization

The primary activator of puberty is the change in negative feedback sensitivity of the HPG system which allows the increased release of GnRH, LH, and FSH, stimulating and increased release of gonadal hormones. This is regulated by leptin and kisspeptin [277]. The kisspeptin neurons in the ARC are "masculinized" by androgens (or high levels of estrogens via the aromatization of androgens during critical period of prenatal development) and regulate to pulsatile release of GnRH in males [214, 278]. At puberty, there is a critical period of sexual differentiation of neural development which leads to sex differences in cognition, behavior, emotionality, social behavior, and the onset of adolescent NDD [60, 279–282]. This involves the reorganization of a number or neural circuits in the hypothalamus, hippocampus, amygdala, and cortex [59, 60, 283, 284]. Sex differences in this "brain remodeling" involve (1) masculinization of the brain by androgens and an increase in neurogenesis in the sexually dimorphic neurons of the stria terminalis and mPOA-SDN of the hypothalamus which is not shown in females [57, 285], and (2) feminization of the locus coeruleus and the hypothalamic neurons that control the LH surge during ovulation [138, 286]. The medial posterior region of the BNST and the LC thus show opposite patterns of sexual dimorphism. The BNST in males is greater in volume and number of neurons than in females (male > female), while in the LC, the opposite is true (female > male) [287].

After puberty, there is a decrease in the number of neurons, dendrites and synapses in the prefrontal cortex while myelination in the white matter increases, and these changes are more marked in female rats than males [279]. A detailed description of the organization of sex differences in the brain at puberty is given by Peper et al. [288]. Ovarian hormones shape cell number and cell group volume female brains during puberty [59]. The increase in estrogen levels at puberty has been correlated with increases in gray matter in the left middle temporal gyrus and with decreases in gray matter in the superior- and inferior prefrontal, orbitofrontal, parietal, as well as temporal cortices. It is noteworthy that numerous changes in gray and white matter in the cortex were correlated with changes in levels of estrogen throughout the lifespans of human females [289]

Sex Differences in Microglia at Puberty

Glial cells play an important role in the sexual differentiation of the brain during the perinatal organizational period and a sex-specific role in the reorganization of the brain during puberty [290]. In adulthood, male microglia have an enlarged soma and have more pro-inflammatory responses than female microglia, while female microglia have a higher phagocytic capacity and higher capacity than male microglia for cell repair and inflammatory control (Fig. 11.7). These sex differences in microglia at puberty may influence the sex differences in the lifespan development of neurodegenerative diseases during later adulthood as well as NDDs [11]. Thus,

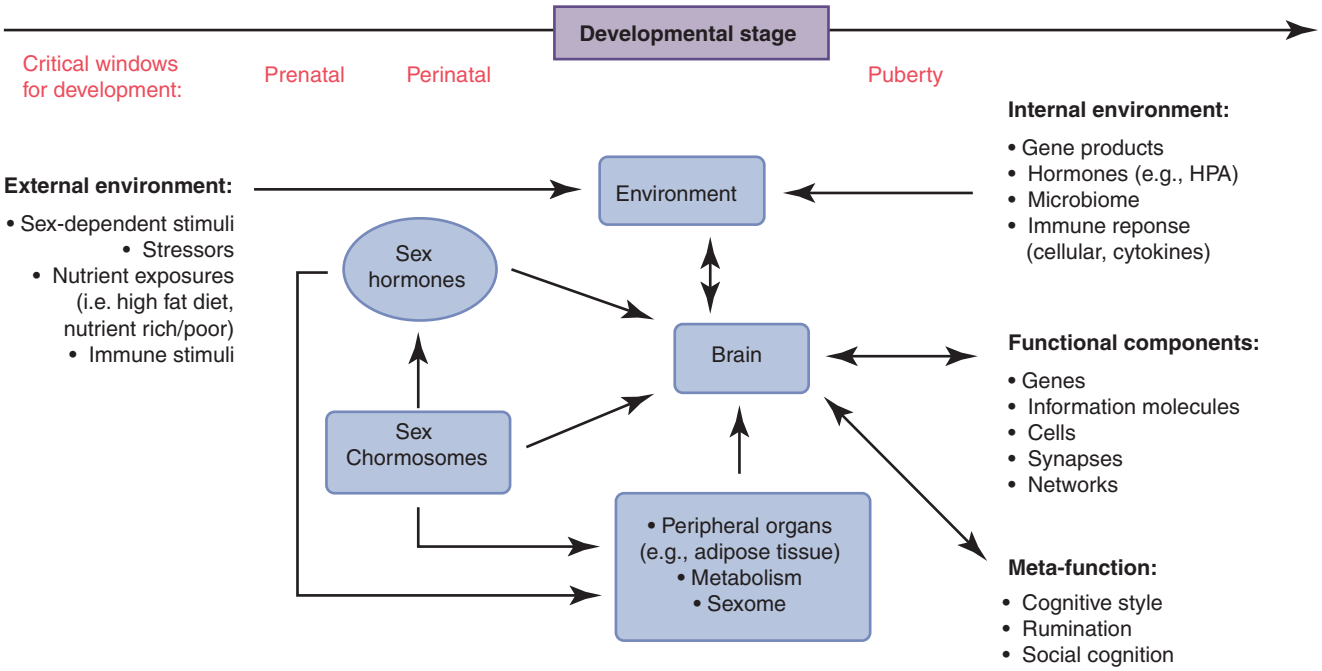

Fig. 11.7 Sex differences in microglia at puberty. A schematic depiction of the multiple levels at which sex influences brain function. The organizational and activational effects of the sex chromosomes and gonadal sex hormones during critical periods of development produce sex differences in brain organization and function. Sex influences the internal environment in which brain function occurs (e.g., differential exposure to stress or immune soluble molecules) as well as modulating the impact of the external environment (e.g., diet or stressors, particularly in the prenatal environment, or even social responses from others based on sex). Sex chromosomes impact brain development directly, may impact physiology through differences in exposure to gene products (e.g., sex-linked genes or differences in gene dosage), and alter brain function developmentally and activationally through sex-determined gonadal function and differential exposure to sex hormones. Sex differences in peripheral organs (e.g., adipose, liver) lead to differential exposure of the brain to hormones as well as medications (through effects on metabolism). The "sexome" refers to the cumulative array of sex-related modulatory effects on intracellular molecular interactions. Sex differences appear at all levels of neural organization, from cell to circuit. Finally, reported sex differences in metacognitions may influence perception and processing of environmental stimuli, thus influencing affective generation and regulation. [From: Rubinow and Schmidt 2019. Sex differences in the neurobiology of affective disorders. Neuropsychopharmacology. 44: 111–128. Figure 1 [228]]

some of the neuroprotective effects of estrogen may involve female microglia. For example, in mice, males show a greater inflammatory response than females to brain injury, while microglia from adult females reduce the damage caused by cerebral ischemia [291]. Microglia have been found to shape sex differences in dopamine pathways at puberty and thus influence sex differences in reward pathways. Microglia and immune-mediated phagocytic activity reduce the number of D1 receptors in the NAc of male but not female rats at puberty [292]. Interactions between glial cells, gliotransmitters, and neurons and neurotransmitters may be essential for the neuroendocrine changes in the hypothalamic-pituitary system at puberty, regulating both the HPT and HPG systems and modulating both metabolism and reproduction [293].

Sex Differences in Gene Expression at Puberty: Epigenetic Effects of Gonadal Hormones

The surges in gonadal hormones during the perinatal and pubertal critical periods are responsible for activating genes that regulate the sex differences in neuroendocrine and neurophysiological activity. In this way, the gonadal hormones act as epigenetic signals to regulate gene expression and this epigenetic modulation of gene expression can be permanent or transitory and can fluctuate across the estrus/menstrual cycle in females [294, 295]. Thus, sex differences in neural activity as the result of gene activation by gonadal hormones can fluctuate over time. This may be one of the many reasons why there is so much variability in measures of sex differences in neuron structure and function: as gonadal hormone levels fluctuate, so does gene expression and neural activity.

Sex differences in gene expression occur during specific critical periods and may be related to sex differences in neuropsychiatric disorders [296]. While DNA methylation at some sites showed sex differences in expression during the critical organizational period, others, which occurred during the perinatal organizational period, were not evident until after puberty [297]. Two genes were of particular interest for their consistent sex-specific expression at different ages: GPR37 (G protein-coupled receptor associated with Parkinson's disease) in females and APLNR (the APELIN G protein-coupled receptor associated with control of the cardiovascular system) in males. Sexually dimorphic genes

involved in synapse formation are also expressed at puberty. Finally, a number of sex-biased genes were shown to be related to NDD. In males these included genes related to autism, bipolar disorder, schizophrenia, Alzheimer's disease, and Parkinson's disease. Female-biased genes were related to OCD, schizophrenia, epilepsy, and AD. Finally, there are sex differences in the activation of gene expression in the PFC, NAc, and VTA of mice in response to stress and cocaine treatment [298]. This indicates a sex differences in gene expression in reward pathways (see below).

Summary

Puberty is both a time when the sex differences in the brain are activated with the rise in gonadal hormones and also a time when a second phase of brain reorganization occurs. While both of these changes depend on gonadal hormones, they involve a number of neurotransmitters and neuropeptides, along with the gonadal steroid hormones, all of which act to regulate gene expression and neuroendocrine development via epigenetic mechanisms of DNA methylation and histone modifications [297]. It is clear that the changes in reproductive function that occur at puberty are accompanied by changes in metabolism, growth, and the functions of the HPA and HPT systems. Puberty is thus a period of neuroendocrine system-wide readjustment which shifts the body from childhood to adulthood. Endocrine disruptors seem to have a more potent effect on puberty in females than in males, but more research has been conducted on puberty in females than males. What is clear is that a wide range of changes in neurochemicals and their receptors occur in the brain at puberty. While kisspeptin seems to regulate the changes in the HPG system at puberty, there are also changes in reward pathways and stress pathways which appear to be sexually dimorphic. All of these changes can result in puberty-related NDDs.

Puberty-Related Neurodevelopmental Disorders

Puberty is associated with a collection of NDDs. These can be divided (arbitrarily) into disorders of puberty timing, eating disorders and addictions, neuropsychiatric disorders, and disorders of sexual development (DSD).

Disorders of Puberty Timing

The most outstanding physiological and physical changes at puberty concern the sexually dimorphic development of secondary sex characteristics. As noted in Table 11.2, there are a number of disorders of sexual differentiation that become obvious at puberty that are due to genetic disorders of sex determination or perinatal hormone disorders of sexual differentiation [299]. The disorders of puberty timing involve precocious puberty or delayed puberty [300, 301]. While these disorders have traditionally been thought of as disorders of the HPG system, it is now clear that disruption of the Kisspeptin system (the Kiss1 gene or the Kisspeptin receptor) is involved in both precocial and delayed puberty as it regulates the release of GnRH [217, 302, 303].

Precocious puberty can be caused by premature activation of the hypothalamic-pituitary gonadal axis (true precocious puberty); by increased estrogen or androgen secretion due to steroid-secreting tumors, or to external steroid hormones in food or vis endocrine-disrupting chemicals (precocious pseudopuberty or Gonadotropin-independent puberty); or to the secretion of gonadal steroids from the adrenal gland (congenital adrenal hyperplasia). The result is that a child goes through puberty at an early age (as young as seven years old in girls or nine years old in boys). Precocious puberty is a sexually dimorphic disorder, being five to ten times more common in girls than boys [300, 301]. Delayed puberty is caused by the failure of the HPG system to develop (hypogonadotropic hypogonadism). In delayed puberty, there is no development of the secondary sexual characteristics. All of the hormones of the HPG system are at low levels, females do not ovulate or show a menstrual cycle and sperm production does not occur in males. Delayed puberty is more common in boys than girls [300, 301, 304].

Sex Differences in the Activation of Reward Pathways at Puberty: Eating Disorders and Addictions

After puberty there are sex differences in addictive disorders, including eating disorders, drug addiction, and other addictions [305] which may be the result of sex differences in neural reward pathways [306].

Reward Pathways The reward pathways in the brain involve dopamine and the opioids and sex differences in these pathways in the NAc and VTA and their pathways to the PFC and the amygdala may mediate sex differences in obesity and addictions [306–308]. Although both men and women show addictive behaviors, women become addicted faster than men, experience more difficulties getting rid of their addictions, and relapse more often than men, and this may be due to the interaction of estrogen and androgens with the dopaminergic and opioid reward pathways [306, 307]. Exactly how sex differences in these reward pathways manifest in addictions is unknown (See long discussion by [306]).

Eating Disorders and Obesity Puberty is one of the most common risk periods for the development of eating disorders, primarily anorexia nervosa and bulimia nervosa, which are more common in girls than boys [309, 310]. In girls the mean age for the onset of eating disorders is between 15 and 19 years of age, but for boys, there are fewer studies and the relationship with puberty is mixed (see [310]). It is possible that perinatal androgens which masculinize the brain reduce the likelihood of the development of eating disorders at puberty in males [309]. There are two ways of looking at sex differences in eating disorders and obesity related to puberty: (1) the close ties between metabolism and body weight with puberty and reproduction in females and (2) the general issue of eating as an addiction related to brain reward circuits. As discussed above, the timing of puberty and the initiation of reproduction in females is closely tied to metabolism and body weight. The development of female reproductive behavior at puberty in rats requires estrogen [74]. The rise in estrogen levels at puberty activates neural reorganization; the female brain is "shaped" by a cascade of hormones and neuropeptides that regulate metabolism and reproduction [289, 311, 312]. The key is the maturation and "rewiring" of hypothalamic neural circuits during puberty. Metabolic signals involving leptin, ghrelin, and insulin regulate the activity of kisspeptin and other neuropeptides regulating the hypothalamic control on GnRH. Thus, any disruption of the metabolic reproductive axis may result in eating disorders [311]. In boys, metabolism and the timing of puberty are less closely intertwined and so eating disorders may be independent of puberty in males.

On the other hand, the conception of eating disorders as addictions focuses on the role of reward circuits in the brain [306, 308, 313]. From this point of view, eating disorders are the result of disrupted dopamine and opioid systems in sexually dimorphic areas of the brain. Because so many components of the reproductive, feeding, and reward systems in the brain are activated at puberty, it is difficult to determine what exactly the term "sex difference" means. It can relate to the sex chromosomes, perinatal organizational period of gonadal sex differentiation, or to the many areas of the brain and neural pathways that are sexually dimorphic (See [314]). In addition, microglia in the medial basal hypothalamus regulate metabolic physiology and may be involved in both metabolic disorders and involvement of metabolism in the timing of puberty [315, 316].

Drug Addiction Drug addiction usually begins between 12 and 17 years of age and although more men use drugs, women are at a greater risk of addiction [305, 317]. Drug use at puberty has been related to sensation seeking and impulsivity in both girls and boys [318] and in women, substance abuse varied over the menstrual cycle [317]. This leads to two hypotheses about sex differences in drug addictions: (1) sexual differentiation of the brain during the neonatal organizational period [317] and (2) the activation/ organization of sex differences in the dopaminergic and opioid reward systems at puberty [319]. Since sex differences in the DA system are due to the effects of gonadal hormones during the perinatal organizational period and the activational period at puberty, the DA hypothesis is a subset of the sexual differentiation hypothesis.

Other Addictions There are many addictions beyond food and drug addiction: sex, gambling, and internet addictions being more common in men, and exercise addiction being more common in women. However, gambling addiction in women may be associated with depression and other psychiatric problems, while in men it is associated with impulsivity, sensation seeking, and risk taking (See [305]). Sex differences in these behaviors may be due to sex differences in reward pathways in the brain or to the activational effects of sex hormones. Other addictions, such as compulsive shopping for clothes (mainly by women) or tools and electronic equipment (mainly by men) and pyromania (mainly by men) may also be related to anxiety, depression, and other psychiatric problems [305], which are also sexually dimorphic (See below).

Sex Differences in Neuropsychiatric Disorders, Anxiety, and Depression at Puberty

Puberty has been associated with a wide range of psychopathologies and neuropsychiatric syndromes in both boys and girls [320, 321]. Twice as many women as men develop anxiety and depression after puberty and it has been proposed that this is the result of the activation of sex differences in the HPA and serotonergic systems at puberty [322]. It is noteworthy that sex differences in cortisol metabolism begin around 11 years of age and increase as puberty advances, after which men secrete more cortisol metabolites than women [323]. However, it has been suggested that the sexual differentiation of the HPG pathway during the perinatal organizational period sets the stage for the activation of sex differences in responses to stress at puberty [324] as there is a close association between the HPG and HPA pathways as discussed above. The sex differences in the development of anxiety, depression and other disorders may be the result of the interaction of stressful stimuli on the sexually dimorphic HPA system [220]. Puberty can also result in the onset of adolescent NDDs, such as schizophrenia [59, 282, 325–327]. Because there is significant synaptic remodeling at puberty, there is the "window of vulnerability" for the development of synaptopathies and for disruption of excitatory: inhibitory signaling ratios. Since the neuroendocrine changes that occur at puberty are all regulated by chemical signaling pathways,

there is the opportunity for environmental epigenetic mechanisms to disrupt the neuroendocrine system during this critical period of brain development [280, 328].

Pediatric Infection-Triggered Neuropsychiatric Disorder Although not particularly associated with puberty, there is a subtype of obsessive-compulsive disorder and/or tics (Tourette's syndrome) which is caused by an infection or neuro-immune activation [320, 329]. This disorder occurs more often in males than females (65:35 ratio) and can begin before or after puberty, with a median age of 11 years old. It has been associated with Group A streptococcal (GAS) infections and the onset is abrupt (e.g., Overnight). As well as OCD it can result in anxiety and/or depression, and sensorimotor disabilities (see [329]). I include it here because of the role of the immune system in neural development and in sexual differentiation in perinatal and pubertal development.

Disorders/Differences of Sexual Development

At one time it was believed that there were only two sexes, male and female, but there can be five sexes or more [330, 331] (Table 11.2). The root causes of the differences in sexual development are a mismatch between sex chromosomes, gonadal hormones, and anatomical features [26, 332, 333]. A true hermaphrodite (Ovotesticular disorder) has both XX and XY chromosomes, both testes and ovaries, a penis and a vagina, and can secrete both androgens and estrogens. A male pseudohermaphrodite (46, XY DSD) has XY chromosomes and testes but a feminized body, with a vagina and breast development due to a lack of androgens or androgen receptors. A female pseudohermaphrodite (46, XX DSD) has XX chromosomes and ovaries but a masculinized body with a penis due to excessive androgen secretion, primarily from the adrenal cortex [332, 333]. There can also be XX males (46, XX testicular DSD) and XY sex reversal (46, XY complete gonadal dysgenesis) (see [334, 335]). These disorders of sexual differentiation are usually detected neonatally. The decision to give surgical or hormonal correction and rear these children as boys or girls is usually made shortly after birth and involves many complex issues (see [334, 335]). What concerns us here are the issues raised at puberty with respect to **gender identity** (whether a person feels that they are male or female), **gender role** (whether a person behaves as a male or female, as defined by their culture), and **sexual orientation** (a person's attraction to the same or opposite sex, or both: heterosexual, bisexual, homosexual). Disorders of gender identity and role fall under the heading of "gender dysphoria."

Gender dysphoria refers to males who identify as females and females who identify as males. Although gender of rearing is the best predictor of gender identity and role in most people with DSD [336] gender dysphoria affects up to 20% of people with DSD [337, 338]. As discussed in Sects. "Sex Determination and Sexual Differentiation" and "Sex Differences in the Brain" on sexual determination and sexual differentiation, females who are exposed to androgens neonatally are masculinized (e.g., congenital adrenal hyperplasia) and males with mutations in androgen synthesis or androgen receptors are feminized [333–335]. Females with CAH have XX chromosomes and are generally reared as female and that works well if the levels of prenatal androgen are low, but if there are high levels of androgens prenatally, the "girl" may decide that she is a boy and wish to have sex reassignment at puberty (See [339]). One procedure to "treat" gender dysphoria in both males and females is to suppress puberty using GnRH analogues, along with psychological support, followed by hormone therapy and gender reassignment surgery. This seems to be successful [340, 341].

Adolescent-onset gender dysphoria is increasing in frequency and is sexually dimorphic, with male-to-female gender dysphoria (transsexualism) having a rate of 6.8/100,000 and female-to-male transsexualism at 2.8/100,000 [342]. Other surveys find that more female than male adolescents report gender dysphoria, with sex ratios of up to 7.5:1 [343]. The "Dutch model" for treating adolescent gender dysphoria is to use puberty suppression around 12 years of age, hormone treatment at 16 years of age, and surgery after 18 years of age [342]. Adolescents with gender dysphoria often have anxiety and depressive disorders, but some are in danger of committing suicide [343]. The phenomenon of "rapid onset gender dysphoria" occurs in adolescents around 16 years of age in more than 80% are female [344, 345]. This is a controversial topic and seems to be a socio-cultural phenomenon rather than a neuroendocrine phenomenon [343, 346]. Time will tell.

Theories of gender dysphoria and the desire for sex change surgery focus on chromosomal sex determination, and hormonal sex differentiation during the neonatal critical period for brain organization. The focus has been on the sexual differentiation of the brain and whether gender dysphoria is the result of abnormal neuroendocrine organization of sexually dimorphic neural circuits in the brain that are activated at puberty. There is considerable evidence that perinatal sexual differentiation of the hypothalamic areas (the BNST and the third interstitial nucleus of the anterior hypothalamus (INAH-3) are two areas which underlie gender dysphoria in males. If they are not sufficiently masculinized by androgens during development, it appears that men may fail to develop a masculine gender identity and role [347–351]. Gender dysphoria is most likely the result of abnormalities in the organizational

effects of sex chromosomes and gonadal hormones during the perinatal organizational period (see [352–354]). Kisspeptin neurons may also be involved in the regulation of sexual differentiation, and the development of gender identity and role and thus dysfunction of the kisspeptin system may underlie gender dysphoria [355]. In addition, the glial cells, which regulate sexual differentiation, metabolism, and the development of the HPG system, may also be involved in gender dysphoria [293, 338, 349, 356].

Summary: Theories About the Causes of Sex Differences in Neurodevelopmental Disorders: Genetic, Hormonal, Immune, and Environmental (epigenetic) Mechanisms

In this chapter I have tried to dissect all of the information on the causes of sex differences in NDDs. From the studies examined, it is clear that (1) sex differences depend on the XX and XY chromosomes; (2) sex differences depend on the gonadal steroid hormones, estrogen, and testosterone; (3) sex differences depend on the sexual differentiation of neurons and neurotransmitter pathways in the hypothalamus and related brain areas: the amygdala, hippocampus, and arcuate nucleus; and (4) sex differences depend on glial cells and gliotransmitters. There is a critical perinatal organizational period for sexual differentiation with masculinization by gonadal androgens occurring before feminization by estrogens. During this critical period many cells in the body are sexually differentiated. Finally, at puberty there is a surge in gonadal steroids regulated by the hypothalamus which activates the sex differences determined perinatally and reorganizes many neuroendocrine brain circuits. Since hormones, neurotransmitters, and neuropeptides act via epigenetic mechanisms to regulate gene transcription in a sexually dimorphic fashion, endocrine-disrupting chemicals in the environment can modulate these same pathways and alter the normal patterns of sexual differentiation during perinatal critical periods.

Finally, it must be remembered that there is the potential for sex differences in gene expression in virtually every cell in the body, including the liver, adipose tissue, muscle, and brain [357, 358]. "Male biased" genes are expressed more highly in males and "female-biased" genes are expressed more highly in females. Most sexually dimorphic genes are located on the sex chromosomes, with more female-biased genes on the X chromosome and more male-biased genes on the Y chromosome, but many autosomes also contain sexually dimorphic gene expression in a tissue-specific fashion [358]. The sex difference in gene expression may be due to the actions of the gonadal hormones as transcription factors. Sex differences in gene expression profiles in brain tissues may underlie the sex differences in neurodevelopmental and neurodegenerative disorders [295, 359]. Likewise, sex differences in gene expression profiles in nongonadal tissues in the body may be associated with sex differences in the incidence of cancer, atherosclerosis, obesity, and responses to drugs [358, 360, 361]. Thus, it is not surprising that there are sex differences in the incidence of NDDs, but it is difficult to pinpoint the causes of such sex differences in individuals. Every patient is their own experiment.

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Multiple-Choice Questions

1. If a person has XXY sex chromosomes, they have the disorder A and will have the physical features of B
   (a) Turner's syndrome; female
   (b) **Klinefelter syndrome; male**
   (c) Klinefelter syndrome; female
   (d) Congenital adrenal hyperplasia; female
   (e) Kallman syndrome; male
2. During the prenatal critical period of development, the hormone A has the effect of B of the brain.
   (a) **Testosterone; masculinization**
   (b) Prolactin; masculinization
   (c) Testosterone; feminization
   (d) Progesterone; feminization
   (e) Corticosterone; masculinization
3. The neuropeptide A regulates the secretion of the hypothalamic hormone B to regulate the sexual differentiation.
   (a) Substance P; GnRH
   (b) Galanin; CRH
   (c) CCK; TRH
   (d) **Kisspeptin; GnRH**
   (e) Kisspeptin; Oxytocin
4. The area of the brain that regulates hormone secretion from the pituitary gland is
   (a) the prefrontal cortex
   (b) the amygdala
   (c) the hippocampus
   (d) the cerebellum
   (e) **the hypothalamus**
5. Bisphenol A is a
   (a) neurotransmitter
   (b) neuropeptide
   (c) **endocrine disruptor**
   (d) hypothalamic hormone
   (e) anterior pituitary hormone

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Part II

Autism Spectrum Disorders

The History of Autism Spectrum Disorder

12

Alexandra Jackman and Lonnie Zwaigenbaum

Highlights

- Factors, such as psychoanalytic theory, developmental theory, neurobiology and genetics, have influenced the understanding and definitions of 'autism'.
- Although the definition of 'autism' has evolved over time with scientific advances and associated social and cultural influences, the core features of challenge in reciprocal social interaction and desire for sameness remained stable.
- Although Leo Kanner is credited with the first description of 'autism' as a distinct condition, multiple forebears and contemporaries explored the concept alongside him.

Introduction

The history of autism is both rich and complex, previously described by clinicians, historians, sociologists, parents and reporters, through diverse lenses. We cannot overemphasize the importance of social factors in shaping historic and contemporary views regarding child development, mental health and the concept of autism as a neurodevelopmental condition. Acknowledging previous excellent reviews that have delved deeply into the social, cultural and political environments that contributed to our current understanding of autism, this chapter aims to summarize the effects of these factors and provide an orientation to the history of autism and its key contributors. Throughout this chapter the terms autism and 'autism spectrum disorder' or ASD will be applied depending on the historical and clinical frameworks in use during the period discussed.

It must be recognized that despite our efforts to simplify the history of autism into a chronological timeline, this approach is somewhat artificial, as the evolution of conceptual frameworks and worldviews has varied greatly with differences in geography and the associated social contexts. Although we describe a sequential progression for simplicity, the evolution can be better represented by an ascending spiral, with concepts building upon previous descriptions, theories and empirical data. While early definitions of autism are different from those we use today, there are consistent themes and ideas that have shaped our views and knowledge of the condition. In this chapter, we try to summarize the evolving understanding of autism, as well as the contexts and information that contributed to the process.

Early Definitions of Autism

Although we now understand autism as a neurobiological condition with its origins related to early brain development, its initial characterization is tightly linked with psychiatric observations of behaviour among adults with mental illness. Eugen Bleuler, a German-speaking Swiss psychiatrist, first coined the term 'autism' around 1908 (in published work, 1911) to describe a feature of patients with schizophrenia. Bleuler credited Sigmund Freud with the etymological roots of the term 'autism', as Freud used it in 1905 to describe an infantile stage of hallucinatory thought to be associated with self-soothing [1]. These are the first known documentations of 'autism' as a behavioural descriptor applied to the human state. 'Autism'[1] is derived from the Greek word 'autos',

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[1]Autos. 2020. In *Merriam-Webster.com*. Retrieved June 24, 2020, from: https://www.merriam-webster.com/dictionary/aut-#etymology

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meaning 'self' and the Greek suffix 'ism,'[2] which may imply an act, a condition, a theory or a characteristic. The origin of the word autism thus indicates a quality of being in, or of, oneself. Bleuler himself defined autism as 'a definite withdrawal from the external world' (Bleuler, quoted in Kanner, 1973: 94) [2]. He described the essence of autism as 'a lack of contact with shared reality' [1].

In Bleuler's early career, schizophrenia was understood to have a biologic aetiology, and it was within this context that the notion of 'autism' originated. However, in the early twentieth century the psychoanalytic theories of Sigmund Freud and colleagues were highly influential. These theories espoused the effects of the unconscious on human behaviour and significantly shaped the period's understanding of human emotion, thought and experience. Within this framework mental health conditions and disorders of psychosis, including schizophrenia, were understood as a reaction to external environmental or relational stresses, as opposed to being biologically derived.

Bleuler's understanding of 'autism' as the idea of withdrawing into oneself was concurrently described by psychologists and psychiatrists across Europe in the 1910s–20s under a number of similar labels including autophilia, egocentricity and ego-hypertrophy [1, 3]. Incorporated into clinical case descriptions, the concept of autism was broadened to include non-pathologic daydreaming and fantasy, termed "autistic thinking" [2]. While Bleuler felt that autism was "an exaggeration of a physiologic phenomenon" [1], and only became pathologic in the context of schizophrenic illness, the word was nonetheless linked with schizophrenia (specifically, hallucinatory thinking) for several decades [1].

Child and Adolescent Psychiatry as an Emerging Field

The move within psychiatry to encompass pediatric mental health emerged from observations of unusual or atypical child behaviour and was contextualized within existing frameworks of adult psychopathology [4]. Henry Maudsley, one of the founders of modern psychiatry, suggested that children with unusual behaviour could be described as having childhood psychoses in 1879 [5–7]. At that time, psychosis was understood as 'organic' in aetiology and having a distinct onset, preceded by typical development and usually, good health.

Subsequently, in the early twentieth century, alongside emerging psychoanalytic theories of mental health, there was a simultaneous interest in the process of child development. Jean Piaget's theory of *cognitive development* was prominent from the mid-1930s to 1960s. *Cognitive development* frames children's learning through their relationships with people and the environment, and Piaget theorized that children constructed knowledge through experience [8, 9].

Thus, multiple frameworks intersected to influence the early understanding of child development and mental health. Notable among these were the longstanding notion of mental illness as being biologically derived, Freud and colleagues' psychoanalytic theories, and Piaget and colleagues' theories of cognitive development. All were fundamental in shaping pediatric psychiatry in the early to mid-1900s.

During this time, clinicians began to recognize subgroups of children with mental illness and atypical development. Because of the historic and linguistic link between the term 'autism' and schizophrenia, the identification and understanding of subgroups of children was shaped by early characterization of schizophrenia. In the first half of the twentieth century, multiple entities that we would now uniquely identify were subsumed into a single category. The category was variably referred to as 'childhood schizophrenia', 'childhood psychoses' and 'infantile dementia' and included children with a variety of differences in development, behaviour, communication, emotionality and thinking. Geographical, cultural and linguistic differences contributed to limited sharing of clinical experience and theoretical perspectives.

Applying Psychoanalytic Theories to Understand Childhood Schizophrenia

During the 1920s–50s, there was a growing interest in understanding the developing infant mind and its relationship to objective reality; that is, how infants developed a sense of self, and how this intersected with relational and environmental factors. It was thought that if a normal developmental process was identified, abnormal variations could be studied as possible causes of childhood psychosis.

As in the adult context, psychoanalytic theories were commonly employed in this area of study. Jean Piaget, a Swiss psychologist who had trained under Bleuler, drew on Bleuler's and Sigmund Freud's use of the term 'autism' to describe an infantile state of egocentrism. In 1922, Piaget presented a paper entitled 'La pensée symbolique et la pensée de l'enfant' (symbolic thought and children's thought), at the International Conference on Psychoanalysis in Berlin, which "linked the concept of autism directly to the child's progressive attempts to engage with reality", [10] and connected infantile thinking with unconscious symbolic thought as described in adult psychoanalytic theory [10]. Piaget, Freud and Bleuler's work influenced child psychologists in Britain who were applying psychoanalytic frameworks in an attempt to conceptualize infants' thoughts. This group included Melanie Klein, Susan Isaacs, Mildred Creak, Anna Freud, John Bowlby and Elwyn James Anthony [10].

[2]Ism. 2020. In *Merriam-Webster.com*. Retrieved June 24, 2020, from: https://www.merriam-webster.com/dictionary/ism

Anthony described an early infantile state of egocentrism, "where the self and the environment are one and there are no permanent external objects", [1, 11] a view shared by Anna Freud and Isaacs [1, 10]. In the 1940s–50s this state of mind was sometimes referred to as 'infant autism'. Some psychologists theorized that children with schizophrenia did not develop typically past this state and thus remained disconnected with the external world [1].

Concurrently, the notion that the maternal state significantly affected infant development was well accepted, and 'maternal deprivation' was thought to have a negative effect on children. This view was based on case series that often focused on children with extreme neglect, social deprivation or environmental upheaval [1, 10, 12, 13]. Many developmental psychologists and child psychiatrists generalized from these extreme cases in their observation of mother–child dyads and concluded that impaired maternal relationships were largely responsible for the affected child's limited capacity for other social relationships and their tendency to "retreat from the outside world" [1] into schizophrenic illness.

Some clinicians criticized psychoanalysts for applying theories of unconscious processes to infants without clear evidence [1], while others wholly embraced the concepts. Bruno Bettelheim, an Austrian trained psychologist who worked predominantly in the United States, was one of the most notorious supporters of environmental effects producing an autistic state, with particular emphasis on the mother-child bond. In the 1950s–70s, the concept of the '*refrigerator mother*'—a parent whose distant emotional state caused their child's 'autistic withdrawal'—was highly popularized, and Bettelheim contributed significantly in the dissemination of this theory.

Categorizing and Classifying to Understand Childhood Schizophrenia

Alongside psychoanalysts' attempts to understand the aetiology of 'childhood schizophrenia', efforts to characterize distinct clinical presentations began in the 1910s and continued into the 1960s, after which empirical approaches were more broadly applied. Children were initially grouped based on features, such as language abilities, atypical behaviour and estimated cognition. In addition to 'autistic', some of the descriptors applied to children who were observed to be withdrawn and have different thought patterns (for example, in imaginative content or topics of interest) were 'schizoid thinking' and 'schizoid personality' [14]. In review of early child psychiatry literature, some of these descriptions overlapped with ASD symptoms as currently described; however, the term autism was not consistently applied.

One of the most important distinctions of early classification frameworks was the delineation between children with an acute onset of symptoms after typical development, compared to those with a more insidious onset. DeSanctis was one of the earliest contributors, describing a phenomenon, 'dementia precox', with loss of language, social and other skills following a period of normal development (DeSanctis 1906; 1908, cited by [4]), not unlike autistic regression. Another key contributor was Thomas Heller, who published a case series in 1930 describing a group of children with typical development, followed by a rapid regression with loss of social, language and other skills which he termed Childhood Disintegrative Disorder [7]. In the late 1930s, Louise Despert, an American psychiatrist, reported case descriptions of children with 'childhood schizophrenia' and divided them into two groups, based on period of onset, recognizing an insidious early onset in some children [15].

Autism Emerging as a Distinct Disorder

Leo Kanner, an Austrian-American psychiatrist, is often credited with introducing 'autism' as a distinct disorder in a seminal paper describing 11 children with an "*anxiously obsessive desire for the maintenance of sameness*" [16] (italics original) and an "extreme aloneness from the very beginning of life" [16]. Kanner's case series was published in English in the United States in 1943. As noted above, there is controversy over whether clinicians before Kanner recognized ASD as now defined but described it in the context of 'childhood schizophrenia', and obtained little recognition. Notable among them is Grunya Sukhareva, a child psychiatrist who published case reports in the 1920s and 30s in Russian and German [17]. She described a group of boys with 'schizoid psychopathy' and 'autistic psychopathy' with symptoms of solitude, psychic inflexibility and motor impairments [17]. Hans Asperger described a similar group of children, though with emphasis on high cognitive capacity as a characterizing feature [18, 19]. He applied the term 'autistic psychopathy', presenting in German in 1937/38 and publishing his thesis in German in 1944. His contribution to the field was largely unknown until it was summarized in English by Lorna Wing in 1981 [20], and later translated by Uta Frith in 1991 [21].

For decades, it was assumed that because of geographical separation and inaccessibility of Asperger's German-language publication, particularly during World War 2, that Kanner's and Asperger's conceptualizations of autism were entirely independent, but recently published historical accounts [22, 23] have recast their relationship, and brought additional contemporary key thinkers into view. Two experienced clinicians and academics, George Frankl and Anni

Weiss, worked closely first with Aspergers, then with Kanner. This suggests that Aspergers' and Kanner's similar vocabulary use and closely timed case series may not have been entirely coincidental.

Kanner and his collaborator Leon Eisenberg credited multiple contemporaries with making observations similar to their own, including Despert, Rank, Weil, Sherwin, Murphy, Cappon, Creak, Stern and Schachter [24]. While Kanner's evaluation was certainly a product of concurrent theories and knowledge in the field, his original 1943 case series was unique in its presentation of childhood behaviour. Instead of "attributing complex unconscious thought processes to children he observed, he simply described the behaviour of a group of children with similar symptoms" [1]. He also eloquently and explicitly described autism as a child's "inability to relate themselves in the ordinary way to people…from the beginning of life" [16], suggesting an innate condition. In these two ways, Kanner differed from predominant psychoanalytic theories of experientially derived illnesses and contributed novel perspective to the field of child psychiatry.

However, even as autism was recognized as a distinct entity by some clinicians as early as the 1930s–40s, there was still much controversy regarding how it fit into the contemporary discourse of child development and mental health. The delineation of autism as a specific condition took time, and in fact, "the diagnoses of schizophrenia, psychosis and autism in children were largely interchangeable" [1] in the United Kingdom and North America until the 1960s. It was not until the third edition of the Diagnostic and Statistical Manual was published in 1980 [25] that autism (formally, 'infantile autism') was widely recognized as a discrete condition. References to autism in earlier editions were limited to describing manifestations of schizophrenia in young children. For example, in DSM-II 'autistic' (not autism) is mentioned only once: "… for cases in which schizophrenic symptoms appear before puberty. The condition may be manifested by autistic, atypical and withdrawn behaviour; failure to develop identity separate from the mother's; and general unevenness, gross immaturity and inadequacy of development" [26].

Differentiating Between Autism as an Intrinsic Condition Vs. Autism as Experientially Derived

Differentiating between autism and childhood psychosis as biological in its origin versus experientially derived was a hotly debated topic between the 1940s and the 1970s. The controversy of 'nature vs. nurture' was immense during that period, and multiple prominent clinicians, including Kanner, oscillated in their views regarding the aetiologic contribution of each [24]. In his original 1943 case series, Kanner recognized both components, commenting on parental emotional and behavioural attributes, while also highlighting the children's "innate inability to form the usual, biologically provided affective contact with people, just as other children come into the world with innate physical or intellectual handicaps" [16]. However, other prominent psychiatrists at the time criticized this perspective and instead argued in favour of relational or environmental effects [13, 27].

In 1949, Margaret Mahler, a Hungarian-American psychoanalyst, co-presented the concept of accepting multiple aetiologic origins of infantile psychoses and classifying them based on a relational aetiology versus an inherent biologic condition, such as 'autism' as initially characterized by Kanner [28]. Mahler herself was interested in relational effects on development and introduced the theory of *separation and individuation* to conceptualize infant cognitive and emotional development, wherein the child transitions from a complete dependent symbiosis with their primary caregiver to independence as an individual [29, 30]. She argued that some infant psychoses and autistic states resulted from variances in this developmental process [28].

Mahler and colleagues thus accounted for the possibility of innate psychosis and autism, while also recognizing the importance of external forces in child development [28]. In 1955, Eisenberg and Kanner re-considered the possibility of environmental factors influencing a child's autistic presentation, observing that "the emotional frigidity in the typical autistic family suggests a dynamic experiential factor in the genesis of the disorder in the child" [24]. It is thus evident that even though Kanner had advanced the theory that autism was innate, the prevailing view of the disorder through the 1950s and 60s discounted biological influences and attributed symptomatology to dysfunctional parenting, to the detriment of many affected individuals and their families.

Applying an Empirical Behaviourist Approach to Understand Autism

In the early 1960s, strengthening empirical and behaviourist perspectives gained momentum, particularly in the UK. Mildred Creak established a working group to enable population-based studies of childhood developmental and psychiatric conditions [1]. The group developed nine key features shared among those affected by the 'schizophrenic syndrome in childhood', generating an early diagnostic framework for autism [1]. Alongside Creak's efforts, a growing number of Britain's prominent academic psychologists and psychiatrists endeavoured to describe all childhood developmental and mental health conditions, including autism, in terms of observable behaviour. Viktor Lotter and Michael Rutter were key thinkers, becoming prominent in the field of child development in the mid-1960s. Their initial

work viewed 'autism' as a behavioural syndrome, as opposed to a specific psychiatric condition. For example, a child's ability to relate to others was characterized in terms of observable behaviours rather than in theoretical terms that relied on inferences regarding the child's thoughts, feelings and relationships [1]. In 1968, Rutter applied this approach to distill Creak's nine key features of the 'schizophrenic syndrome in childhood' into three key features: 'profound abnormalities of language development, a variety of ritualistic and compulsive phenomena ... [and] a particular variety of disturbance in interpersonal relationships' [1].

Further elaboration of these features formed the basis of the first formal diagnostic criteria for autism (formally, 'infantile autism',) in the third edition of the Diagnostic and Statistical Manual [25]. Throughout the mid-1960s to early 1990s empiric study has provided numerous insights into the common features, prevalence, co-morbidities and aetiologies of autism. Particularly in the 1960s–80s, Rutter and colleagues in the UK contributed substantially to this increased understanding. Their application of empirical methods to investigate behaviour, cognitive, language and social abilities has shaped the current understanding of ASD as a neurobiological condition.

Exploring Autism as a Language Disorder

As investigators focused on quantifiable observations and argued against inference of thought content based on a child's behaviour, language ability emerged as a characteristic feature among groups of children, and an increased area of study. Rutter and colleagues conducted longitudinal follow-up studies of children diagnosed with 'early onset psychosis' and found that though the children displayed unusual behaviour and language characteristics, there was no convincing observational evidence or individual report of hallucinations [1]. Israel Kolvin further investigated individuals with childhood psychosis, applying stringent inclusion criteria and empiric variables to categorize groups of children. He found that children who presented with 'psychosis' under 3 years old were significantly more likely to have severe speech delay. Kolvin's rigorous statistical studies were important in shaping the concurrent understanding of autism as a language disorder, as they were highly reproducible. In 1971, Kolvin's findings reaffirmed Rutter's 1968 '3 key features' when he reported that children under 3 years with 'psychosis' were most likely to present with a triad of symptoms, including severe speech delay and/or speech anomalies, stereotyped movements and poor relationships. These findings cemented the understanding of autism as separate from schizophrenia and also linked it to language development [1].

Throughout the late 1960s–70s researchers investigated the linguistic patterns of children with autism. The language skills of children with autism were compared to those in children with language disorders, such as aphasia, and sensory disorders, such as visual impairment and deafness, in a search for reliable identifying features. In the mid-1970s Lawrence Bartak, Anthony Cox and Rutter confirmed longstanding case report findings of distinct language use [16, 17, 21], reporting that a subgroup of children with autism shared specific language patterns, including echolalia, pronoun reversal, stereotyped utterances, repetition of phrases and odd intonation [1]. Further investigation of language and cognitive abilities by Frith, Hermelin and O'Connor identified specific areas of impairment including grammatical rules, gesture and semantic word association. In this period many researchers conceptualized autism as a disorder of specific cognitive and linguistic deficits [1, 2].

Exploring Autism as a Social Cognition Disorder

Despite the established relationship between autism and language, many clinicians and researchers felt that a conceptualization of autism as a linguistic disorder did not account for the "heart of the matter – namely the presence of an impaired capacity for human relationships" [31] as described by Mildred Creak in 1961. Although social difficulties could not be solely attributed to language development, they remained under-studied until the 1980s. During this period researchers collaborated across countries and disciplines in the translation of basic science research of social development in human and primate infants to the exploration of social deficits in autism [1, 32]. This translational research generated the thesis that disordered social cognition, specifically, 'Theory of Mind' deficits, was foundational to autistic symptoms. The term 'Theory of Mind' was drawn from D. Premack and G. Woodruff's research on chimpanzee social behaviour and cognition. It was defined as the ability to "to infer the full range of mental states (beliefs, desires, intentions, imagination, emotions, etc.) that cause action… to be able to reflect on the contents of one's own and others' minds" [33].

In 1985, Simon Baron-Cohen, Alan Leslie and Frith compared autistic children to typically developing children and children with trisomy 21 (and below average full scale IQ), identifying that the children with autism had a unique relative weakness in their ability to "impute mental states to oneself and to others" [34], compared to a relative strength in cognitive ability [34]. This seminal paper brought social cognitive frameworks into the forefront of understanding behavioural features of autism. It was argued that theory of mind deficits could account for several of the specific social defi-

cits observed in ASD. For example, reduced shared attention (nearly universal in young children with autism) could be related to poor awareness of others' mental states, whereas social approach, which is more variable, does not require such awareness [35]. Similarly, individuals with autism with age-appropriate vocabulary and grammar nonetheless displayed difficulties using and interpreting metaphorical language, lacking a shared understanding of how others understand non-literal expressions [36].

It was recognized, however, that theory of mind deficits could not easily account for other features of autism, particularly restricted interests, repetitive behaviours and insistence on sameness. Moreover, some children with ASD were able to pass both simple ('first-order') and more complex ('second order') theory of mind tasks (reviewed in [35]). Additional cognitive theories of ASD were proposed to account for other common features of autism. These included deficits in executive function (explaining difficulties with planning/organizing and pattern recognition) [37] and weak central coherence (difficulty processing information for global meaning, e.g. face processing/recognition of facial emotion) ([38]; cited in [35]). The central coherence theory was recognized to have implications for strengths on tasks that rely mainly on recognition of component details rather than a coherent whole. For example, the 'Block Design' subtests on a standard cognitive assessment [39] and to the presence of exceptional abilities, for example, in music perception, including perfect pitch [40]. Happé, Ronald, & Plomin [41] subsequently argued that based on relative genetic independence of autistic traits (e.g. social impairments vs. repetitive behaviours), and the failure of any single cognitive mechanism to account for the full range of symptoms, that it was 'time to give up on a single explanation for autism'. However, theory of mind, executive function and the balance between strengths in detailed-oriented vs. holistic processing continue to inform understanding of the diverse manifestations of autism.

Early Understandings of Autism as a Neurobiologic Condition

One of the earliest advocates of a non-psychogenic aetiology of autism was Dr. Bernard Rimland, an American psychologist and parent of an autistic child. In 1964, he published *Infantile Autism: The Syndrome and its Implications for a Neural Theory of Behaviour* [42], proposing that autism was most likely multifactorial in aetiology with genetic, neurologic and environmental contributions. The 1970s and 1980s saw an increasing conceptualization of autism as a neurobiologic condition, with significant relationships to well-accepted neurologic co-morbidities and heritability confirmed empirically. These studies provided indirect evidence against a psychogenic aetiology of autism by linking it to well understood biologic concepts. We will highlight a small selection of seminal studies that shaped this view, as further elaboration on the genetic and environmental influences and the developmental neurobiology of ASD is included in subsequent chapters of this textbook.

The aetiologic relevance of prenatal environmental exposures was first established empirically in the aftermath of the 1964/65 congenital rubella epidemic in the United States, where an estimated 20,000 infants were born with congenital rubella syndrome [43]. Stella Chess, an American psychiatrist, determined that the prevalence of autism was significantly higher among children affected by congenital rubella [44], linking the condition to neurologic injury. Other early evidence implicating neurological abnormalities in autism included the longstanding observation of increased prevalence of epilepsy among children with autism, affirmed by Deykin and MacMahon [45] and others in population studies. It was reported that about one-third of children with autism developed epilepsy, the natural history of which was relatively unique, with bimodal onset in the preschool and adolescent years [46].

Increased head circumference was noted by Kanner in five of 11 children in his original case series [16] although was not given much initial attention. The finding did not reappear in the research literature until the 1970s, when growth parameters were included in measures of 'minor physical anomalies' [47, 48] aimed at implicating 'organic factors' in children with psychosis and other behavioural disorders and subsequently applied to the study of autism (e.g. [49]). Identifying such physical anomalies was intended to help identify potential aetiologic factors but was not ultimately informative in that regard. However, there was increased interest in macrocephaly as a biological marker for ASD, with additional reports in the 1980s and 1990s (e.g. [50, 51].), and the suggestion that enlarged head size observed at diagnosis might represent abnormal post-natal growth [52]. Subsequent research has been less conclusive, with inconsistent findings among studies that included community controls rather than published (and likely outdated) norms [53] and from population-based birth cohorts [54]. However, studies of head circumference have led to MRI studies of brain growth, which have shown impressive evidence of increased brain volume associated with ASD early in life in cross-sectional [55, 56] and more recent longitudinal studies, which have implicated increased cortical surface area in the first two years of life [57].

While the possibility of heritability had been explored since Kanner's original case series, when he commented on high parental education level and "a great deal of obsessiveness in the family background [16]", such familial traits were largely interpreted in the context of early parent–child interactions in subsequent decades. It was not until the pub-

lication of the first twin study in 1977 that evidence of genetic contribution was confirmed. Folstein and Rutter [58] studied 21 twin pairs and found higher concordance of autism among monozygotic than dizygotic twins. This landmark study specifically implicated a strong genetic contribution to aetiology and ushered international efforts to identify specific genes using increasingly sophisticated methodologies, reviewed in detail in Yuen et al. in Chap. 18. The initial focus was on specific syndromes with elevated rates of autism, such as Fragile X and Tuberous Sclerosis [59] and genome-wide linkage studies [60] complemented by studies mapping candidate genes at breakpoints identified in cytogenetic studies [61, 62]. Subsequent genomic studies of rare copy number variants were enabled by high throughput sequencing techniques [63, 64]. Whole genome and exome sequencing studies, combined with cellular and animal models, have yielded important insights into the potential neurobiological mechanisms underlying the development of ASD (see review by [65]).

Research on genetic contributions and related biological mechanisms has also focused attention on sex-related differences in autism rates and associated phenotypic profiles, with important implications for both underlying biology, as well as concerns that biases in clinical practice may lead to under-identification of ASD in girls and women (reviewed in [66] and in Chaps. 11 and 18).

Looking Back: Possible Early Accounts of Autism in our Histories

In the 1980s–90s, as academic interest in autism grew and epidemiology studies demonstrated increasing prevalence, scholars reviewed historical documents and oral legend searching for early accounts of the condition. For example, Wing [4] noted that Brother Juniper, a follower of St. Francis of Assisi, and the 'old fools of Russia' are described as being socially naïve and misunderstanding the intentions and expectations of others, thus demonstrating what would later be described as social communication challenges inherent to autism [2, 4, 38]. In European and Indian history, there are multiple accounts of 'wolf children' witnessed to survive in the wild independently with limited language and social skills [7]. The most famous of these is Victor, 'the wild boy of Aveyron' found in the French forest in 1798 and rehabilitated by Dr. Jean Itard. Victor was described as "expressionless, with limited eye contact, overly focused on desired objects and had no speech" [7]. His strengths were a good memory and sense of order, and with Dr. Itard's care Victor learned to distinguish emotions, develop affection for people and acquire rudimentary reading and writing skills [7]. There is also written documentation in Scottish law records of an individual named Hugh Blair, who lost his land rights on the basis of limited mental capacity. Blair was described as socially tactless, having poor eye contact, echolalia, insistence on routine and odd interests. Blair's case in 1747 identified an early record of autism symptoms documented in a person living with otherwise typical environmental conditions, unlike 'feral children' (such as Victor) who grew up with little social contact [7]. Possibly the earliest medical record of autism is within John Haslam's 1809 publication "Observations on Madness and Melancholy" wherein he described a case of a boy with solitary play, pulling to gain attention, referral to himself in the third person and a number of ritualistic behaviours, including attending church without specific purpose and only voiding in a bowl (i.e. urinal), despite this being atypical for the situation [7].

More recent authors, such as historian Berend Verhoeff, have challenged the utility and validity of retrospective ASD diagnosis [2], and clinician-historians have also recognized the intrinsic complexity of the enterprise [7]. Nonetheless, over the past thirty years the history of autism has evolved into an independent area of study, contributing to our current definition of ASD as a scientific community, as well as within the broader public. As clinicians, an awareness of this history, particularly cultural influences, allows us to critically evaluate evidence, as well as negotiates biases during assessment and diagnosis in clinical encounters.

Other Social and Cultural Factors Shaping Views of Autism

Finally, we will highlight other important social and cultural factors that have contributed to the understanding and societal perceptions related to autism.

A major contributor to how autism and other developmental conditions are characterized and perceived has been the *physical location* of affected individuals. Historically, persons with developmental differences have been institutionalized across various cultures and contexts. Public and private institutionalization was a dominant practice in the first half of the 1900s in North America, Australia and areas of Europe for children with behavioural, cognitive and psychiatric challenges [67]. Institutionalization has been criticized as perpetuating the marginalization of individuals with developmental differences, and by the 1960s a 'community living movement' grew. It aimed to bring institutionalized individuals into the community, with continuity of support services [68]. With the transition to community living local schools were tasked with providing education to individuals with developmental and behavioural differences. This lead to increased employment of educational psychologists and speech and language pathologists in schools [1]. Both disciplines brought new lenses to the understanding of children with developmental differences, particularly autism,

throughout the 1960s and 1970s, which was translated to the academic community [1]. A parallel process of de-institutionalization and integration occurred in multiple countries in Europe, Australia and North America, although the timelines of the history are variable [1, 67, 68].

Parents, who for many years were often marginalized by clinical paradigms that attributed children's impairments to their interactions and minimized their role in intervention programmes, have played a critical role as self-organized advocates since the 1950s. Concurrent with de-institutionalization, the first national autism parental advocacy groups were established in the early 1960s (UK Society for Autistic Children est. 1962 [1] and The Autism Society of America est. 1965—then called the National Society for Autistic Children) [69]. These organizations lobbied government and contributed to the development of legislation for non-discrimination in accessing federal assistance and education, including the Education for All Handicapped Act (EHA) in 1975, (reauthorized in 1990 as the Individuals with Disabilities Education Act, or IDEA) and the Americans with Disabilities Act (1990) [69]. These organizations served as points of connection for families with information, support services, physicians and other families.

Several mothers of children with autism were particularly strong voices in the early history of autism advocacy, including Dr. Ruth Christ Sullivan who contributed significantly to the above legislation, and became the first elected president of the Autism Society of America in 1968 [69]. Rosalind Oppenheim increased public awareness of the parenting journey for families with autistic children when she published an article in the American periodical *Saturday Evening Post* in 1961. The article, entitled, "They Said Our Son Was Hopeless" resonated with families across the United States, and Oppenheim became a beacon for those on similar journeys [69]. Oppenheim's son was rejected from multiple schools in her Chicago area, motivating her to co-found The Rimland School for Autistic Children in 1971, a school and transition service for individuals with autism that continues to service the community today [70]. Clara Claiborne Park was another motivating force in the United States. She published *The Siege* [71], the first parent-authored book illustrating the journey of raising a child with autism, providing other families with a resource and access to a shared experience [23]. All three women rejected the dominant theory of the 'refrigerator mother' as the cause of autism and advocated for different research approaches and education for children with autism.

Like these mothers, Dr. Bernard Rimland rejected psychoanalytic theories and was also a pioneer in consideration of autism as a neurobiologic condition, publishing *Infantile Autism: The Syndrome and its Implications for a Neural Theory of Behaviour* [42] in 1964. Rimland was a parent of a child with autism, as well as a psychologist, and became a link for many families to autism research and treatment. He co-founded the Autism Society of America and advocated for a national autism research programme. Rimland's contributions as an advocate were highly impactful, but his legacy also includes active promotion of a wide range of biomedical treatments without empiric evidence and endorsement of unproven theories of autism aetiology, such as risk related to vaccination ([72]; see below).

The parental advocacy tradition evolved to include individuals diagnosed with autism themselves. Dr. Temple Grandin emerged as an important public figure with the publication of her first book, *Emergence: Labeled Autistic*, which illustrated an 'insider's perspective' of autism [73]. In 1988 she was the first autistic person elected to serve on the Autism Society Board of Directors. She continues to write, speak and lobby for inclusion of autistic persons across social contexts and promote the concept of neurodiversity. The term 'neurodiversity' was coined by Dr. Judy Singer [74] in the mid-1990s to capture the concept of variety in neurological development as being a natural part of the human species, akin to sexual orientation or race [75]. She created a name for an emergent cultural and civil rights movement among individuals described as 'high-functioning autistics' or identifying as having Asperger's syndrome who were striving for recognition of the inherent value in autistic singularities [75]. Journalist Harvey Blume popularized the term by exploring its concept in a story called "Neurodiversity: On the neurological underpinnings of geekdom" published in *The Atlantic* in 1998 [76]. The term has been controversial among autism advocacy groups, as some argue that its broad application to highly affected children with ASD is problematic, minimizing their challenges and possibly limiting access to supports [75]. Regardless of the debate over its application, the concept of neurodiversity has had a profound cultural effect on frameworks applied to understand individuals with 'disability' and developmental differences, as well as empowered and connected individuals and families with ASD.

The history of cultural influences and autism advocacy is explored in more depth in numerous publications, and we direct you to the resources below for further reading:

- Silverman, C. (2011) Understanding Autism. Princeton, NJ: Princeton University Press.
- Donvan, J. and Zucker, C. (2016). In a Different Key: The Story of Autism. New York, NY: Crown Publishers.
- Eyal, G., Hart, B., Onculer, E., Oren, N. and Rossi, N. (2010) The Autism Matrix. Cambridge: Polity.
- Evans, Bonnie. (2017). The Metamorphosis of Autism: A History of Child Development in Britain Manchester (UK): Manchester University Press.

Conclusion

The history of autism (now, 'autism spectrum disorder' under DSM-5 and ICD-11) is characterized by a series of transformational changes since the condition was introduced as a distinct clinical entity in the scientific literature less than a century ago. While the core features described in [16] case series show remarkable continuity with current diagnostic criteria, the shift in prevailing aetiologic models from psychodynamically oriented theories to evidence-based neurobiological approaches has been revolutionary, although many fundamental questions about causation and specific mechanisms underlying clinical expression remain. There has also been a marked evolution in understanding of symptomology related to efforts to identify core neuropsychological deficits, complicated by the remarkable clinical and aetiologic diversity. Importantly, parents, who were initially marginalized by early psychodynamic paradigms, are now recognized and valued as essential partners in intervention and ongoing care. Their strong leadership has positively influenced public policy and societal views of ASD. Autistics themselves have also become increasingly empowered as self-advocates and community leaders, although much work is still needed to ensure that individuals across this diverse spectrum are afforded opportunities needed to participate fully in societal roles [77]. With advances in genetics, neuroscience and more fulsome partnerships between clinicians, scientists, autistics and their family members, it is hoped that the next chapter in autism's history will be marked by a deeper understanding of its biological underpinnings, improved diagnostic methods, more effective and individualized treatments, and inclusive communities that support the full range of opportunities for autistics to engage and contribute throughout their lives.

Considering Historical Figures: Spotlight on Bruno Bettelheim

Bruno Bettelheim immigrated from Vienna, Austria to Chicago in the US in 1939. He worked initially as a research assistant and college teacher. He published a paper based on his experiences while detained in Buchenwald and Dachau concentration camps, '"Individual and Mass Behavior in Extreme Situations" [78], which brought him national attention. He secured a position at the Sonia Shankman Orthogenic School, where he served as director from 1944 to 1973, and also had an academic appointment in the Department of Psychology at the University of Chicago. Under his direction, the Orthogenic School served as a residential programme that immersed children with developmental differences in a 'total therapeutic milieu', separated from their parents [79]. He advocated for the psychoanalytically based 'psychogenesis theory' of autism, which attributed the social withdrawal of autistic children to inadequate emotional support from parents (mainly mothers) during infancy. In his writings, he argued that the emotional trauma of parental rejection was analogous to the "extreme situations" that traumatized and profoundly impacted the social behaviour of persons he had observed in concentration camps. His peer-reviewed academic publications as well as popular media presence which included numerous books (e.g. 'Love is not Enough: Treatment of the Emotionally Disturbed Child', [80]), magazine articles (e.g. 'Joey: A Mechanical boy, Scientific American; [81]) and television appearances were characterized by vivid case descriptions that were influential in bringing autism into the public eye. Bettelheim is perhaps best known for authoring "The Empty Fortress: Infantile Autism and the Birth of the Self" [82] wherein he describes several children whose withdrawal and disconnectedness he attributed to rejecting and emotionally distant parent, the so-called 'refrigerator mother' theory of autism aetiology.

The Orthogenic School residential programme itself was not particularly unusual in concept for the time, as the majority of children with developmental differences were institutionalized in North America and parts of Europe. However, Bettelheim's treatment model was founded on establishing relationships between children and their individual counsellor, with the exclusion of parent involvement, including visits for extended periods of time (up to years). Moreover, Bettelheim's conduct in the school has been questioned with multiple reports emerging after his death in 1990 of prior students alleging abuse [83]. While some students, teachers and counsellors have defended their experience, there is nevertheless agreement regarding his use of corporal punishment [79, 84].

Moreover, Bettelheim's credentials have also been brought into question. Investigations into his educational record differ as to whether his PhD was in Art History or Philosophy; some authors (e.g [85].) have concluded that there is no evidence that he received doctoral training in Psychology, with the exception of introductory courses that were the standard requirement for any PhD student at Vienna University [85]. With the shift to biologically oriented views, Bettelheim's proposed aetiological theories, which invariably blamed parents of causing their children near-irreparable emotional harm, were recast as a dark chapter in autism's history.

Considering Historical Figures: Spotlight on Hans Aspergers

Hans Aspergers (1906–1980) was a paediatrician who joined the Vienna University Children's Clinic in 1931 and started working at the clinic's Heilpädagogik (child psychiatry)

ward in 1932, eventually becoming its director. His case series of four children 'Autistic Psychopathy in Childhood' was published in a German language journal in 1944. The children shared commonalities, such as typical language milestones but atypical social communication, poor understanding of social norms, obsessive interests, resistance to change and poor motor coordination.

In 1981, Lorna Wing, a British psychiatrist and pioneer in the autism field, recognized the similarities and differences between Aspergers' case series and Kanners original description of autism. She introduced 'Asperger's syndrome' to the broader clinical community, and as a result, 'Asperger's Disorder', was included alongside 'Autistic Disorder' in the DSM-IV as subtypes of pervasive developmental disorder [86].

Through the early 2000s, Aspergers was lauded for his scientific contributions and his prescient recognition of the potential contributions of individuals with the social and behavioural differences associated with 'autistic psychopathy' [21]. Moreover, early historic and journalistic accounts were suggested that Aspergers was a physician that had worked effortfully to protect his patients as he worked in Nazi-occupied Austria from 1938 to 1945. However, his legacy was profoundly reframed by a detailed historical analysis by Czech [87]. Czech's work provided a thorough historical analysis of Asperger's writings, as well as archival documents, including Asperger's personnel files and his clinical assessment notes, documents which had mistakenly been assumed to have been destroyed in the war. In an accompanying editorial, Baron-Cohen and colleagues [88] commented that Aspergers had "contributed to the Nazi eugenics program by referring profoundly disabled children to the *Am Spiegelgrund* clinic". Baron-Cohen and colleagues concluded that "Asperger was not just doing his best to survive in intolerable conditions but was also complicit with his Nazi superiors in targeting society's most vulnerable people". These findings reframe him as complicit in targeting the same vulnerable children that he was initially viewed as valuing.

Considering Historical Figures: Spotlight on Bernard Rimland

Dr. Bernard Rimland was an American psychologist in a unique position as a researcher and the parent of a child with autism (Mark), sitting at an intersection between two worlds he contributed to significantly. As a researcher, he presented a novel endorsement of a biologic aetiology of autism with his first book in 1964. He maintained this view throughout his career and co-founded the Autism Research Institute (ARI) in 1967 (then called the Institute for Child Behavior Research), with the aim of filling a gap he recognized in autism research [89]. The institute's "mission was to conduct, sponsor and fund research, and to network clinicians, researchers and parents" [89]. However, Rimland and the ARI became increasingly controversial from the 1980s onward when both supported research considered 'pseudoscience' by many contemporary colleagues. Rimland was criticized for endorsing inadequately studied treatments of autism, such nutritional therapies, as well as supporting unproven compounds as causative of autism, such as mercury-containing vaccines [90].

Rimland was a prolific speaker, engaging in more than 1000 talks in his career, writer, publishing over 300 works, and an international advocate. Furthermore, he promoted public knowledge of autism using video media in his 1967 documentary, *Infantile Autism: Behind the Wall*, as well as by serving as a consultant for the 1988 film, *Rain Man* [89]. Rimland died in 2006 and is remembered as "a pathfinder and tireless advocate for families dealing with autism" (F. Volkmar in [90].

Multiple Choice Questions—Correct Answers Bolded

(1) Who of the following were key ASD advocates?
   (a) Bernard Rimland
   (b) Ruth Sullivan
   (c) Temple Grandin.
   (d) **All of the above.**

(2) Which of the below theories implicated parents as the cause of their children's ASD?
   (a) Empirical behaviourism
   (b) **Refrigerator mother theory**
   (c) Psychoanalytic theory
   (d) Attachment theory

(3) Which of the below organic processes was linked to ASD in early population studies?
   (a) Poverty
   (b) Congenital syphilis
   (c) **Congenital rubella**
   (d) Autoimmune diseases

(4) Which of the below factors were early indicators supporting a neurobiologic aetiology of ASD?
   (a) Increased incidence of epilepsy.
   (b) Increased incidence of macrocephaly.
   (c) Increased rates of ASD concordance in monozygotic compared to dizygotic twins.
   (d) **All of the above.**

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Epidemiology

13

Mayada Elsabbagh

Learning Objectives

- Identify relevant international policy frameworks relevant for the epidemiology of autism.
- Summarize available prevalence estimates of autism around the world and their variation within and across geographic regions.
- Identify the methodological features that contribute to under- or over-estimation of true prevalence.
- Critically appraise relevant evidence linking prevalence estimates to risk factors for autism.

Highlights

- Prevalence estimates are essential for informing public policy and raising awareness.
- Epidemiological studies have tracked the prevalence of autism since the 1960s–70s, when autism was thought to be a rare disorder with narrow clinical criteria.
- The clinical definitions of autism have expanded over time to include milder forms and a spectrum of impairments in social communication and behavior. In parallel, considerable progress has been achieved in increasing autism awareness and public health response worldwide, also impacting prevalence.
- The prevalence of autism is around 1–2% of the general population worldwide, but estimates vary substantially within and across geographic regions, reflecting differences in community awareness and service capacity, and patterns of help-seeking, among other factors.
- Although several hypotheses have linked variations in prevalence with risk factors for autism, relevant evidence is absent or limited to date; further research with large representative samples with comparable methods is needed.

Introduction

Various forms of autism affect 52 M children worldwide [1]. The condition emerges in early childhood leading to social and communication impairments across the lifespan. Currently, autism is defined on the basis of social and communication impairment and repetitive and restrictive behaviors that can vary in individuals along a continuum of severity [2]. A diagnosis of autism can be made as early as 18–24 months of age; it is around this age that characteristic symptoms can be distinguished from typical development and from other delays or other developmental conditions.

Advances in autism research have gone hand in hand with significant progress in international policy context. In addition to the policy response resulting from the significant increase in awareness and advocacy worldwide, autism has also benefited from progress in relevant areas, including human rights, maternal and child health, and mental health [3–6].

Against this overall progress in autism research and policy, epidemiological studies have offered objective indicators of the impact of autism, including estimates of cases and their associated social and economic impacts. It is well known that the epidemiological estimates of autism are highly impacted by variations in methodological features of different studies; this includes population and sample characteristics as well as case definition, identification, and evaluation. Although it is well known that methodological

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variation can often lead to over- or under-estimation of prevalence, the utility of these estimates is not always diminished [7].

Even when imprecise, epidemiological estimates are useful tools for advocacy and policymaking because they can reflect the state of identification, services, and supports offered to the affected population. For example, when an epidemiological estimate falls in the lower range of those available worldwide, this would signal to policymakers the need for more services because true cases are not being picked up in routine systems [7–9]. Recently, the surge in epidemiological studies conducted in previously under-represented regions (represented in Table 13.1) has served to increase community capacity to identify autism by supporting development and/or validation of tools and supporting training of clinical/research teams. Therefore, the major progress already achieved in global advocacy and international policy for autism has been informed by existing estimates while simultaneously highlighting gaps in knowledge and community capacity that motivated further research and worldwide action.

In contrast, significant scientific, clinical, and social challenges arise when epidemiological data are used, often indirectly, for inference about etiological risk factors. There are two main classes of such causal hypotheses; the first class relates to time trends where it is proposed that the global increase in autism prevalence reflects a true increase in incidence due to changes in risk factors over time. The second class relates to the interpretation that the variation of prevalence linked with geographic, ethnic, social, or economic factors reflects the true variation in underlying biological and/or environmental risk. Erroneous inferences about etiology from epidemiological estimates has led to a longstanding hype in a cause of an increase in incidence due to increased environmental risks over time without direct evidence to support such claims [10]. It has also motivated research investments into the investigation of specific populations, such as Somali immigrants in the United States, where differences in prevalence were presumed but never corroborated [11]. Finally, limited attention has been paid to investigating competing hypotheses related to health disparities, where stigma and/or systemic barriers leading to marginalization of ethnic or socioeconomic subgroups modify their access to services and as a result lead to a variation in prevalence.

The current chapter illustrates recent progress in the field of autism epidemiology against a background of advances made in awareness and improved international public policy. The next section offers an overview of the international policy context, including relevant frameworks for human rights, maternal and child health, mental health, as well as autism-specific policies. The following section provides an up-to-date review of epidemiological surveys of the prevalence of autism, discussing the methodological features that can lead to over- or under-estimation of true population prevalence. The final section is a critical review of hypotheses linking epidemiological data to risk factors.

Table 13.1 Selected epidemiological estimates of autism worldwide

Year	Authors	Country	Sample size	Age (years)	ASD cases	Diagnostic criteria	Percent with ID	Sex ratio (M:F)	Prevalence/10,000	95% CI	Years studied
2022	Shenouda et al.	United States	26,083	8	942	DSM-5	25.2	4.3	360	340–380	2016
2021	CDC	United States	220,394	4	3,754	ICD-9 or ICD-10	51.6	3.4	170	165–176	2018
2021	Arun and Chavan	India	8,451	1.5–10	19	CASI | ADI-R | CARS-2 | DSM-5	–	1.11	22.5	6.9–51.9	2015–2017
2021	CDC	United States	220,281	8	5,058	ICD-9 or ICD-10	35.2	4.2	230	223–236	2018
2021	Roman-Urrestarazu et al.	United Kingdom	7,047,238	5–19	119,821	ICD-10 | registry-reported	18.08	4.32	176	176–177	2017
2021	Underwood et al.	Wales	2,385,156	All	12,089	ICD-10 | Read codes v2 | registry-reported	–	3.71	50.68	49.79–51.59	2016
2019	Delobel-Ayoub et al.	France	15,836	8	115	Registry-reported	–	5.4	73	60–87	2015
2019	Delobel-Ayoub et al.	Iceland	13,551	7–9	363	Registry-reported	20.9	4.4	268	241–296	2015
2019	Pérez-Crespo et al.	Spain	1,326,666	2–17	15,466	ICD-9	–	4.5	123	121–125	2009–17
2019	Alshaban et al.	Qatar	133,781	6–11	1,099	DSM-V	–	4.26	114	89–146	2015–18
2019	Mohammadi et al.	Iran	31,000	6–18	37	K-SADS-PL	70.3	2	10	9–17	–
2019	Hong et al.	South Korea	51,529,338	0–89	5,653	ICD-10	–	5.4	1.09	1.07–1.11	2015
2018	Diallo et al.	Canada	16,940	1–17	207	ICD-9 | ICD-10	–	3.8	122	104–136	2014–15
2018	Arora et al.	India	3,964	6–9	44	INDT-ASD	–	–	140	60–320	2011–12
2018	Akhter et al.	Bangladesh	5,286	1.5–3	4	MCHAT | ADOS | DSM-IV		3	7.5	–	2016
2016	Fombonne et al.	Mexico	12,116	8	36	DSM-IV-TR | SRS | ADOS | ADI-R	30.5	4.1	87	62–110	2011–12
2016	Chinawa et al.	Nigeria	721	3–18	21	Parent report	–	1	290	170–412	2014

DSM Diagnostic and Statistical Manual of Mental Disorders, *ICD* International Classification of Diseases, *SRS* Social Responsiveness Scale, *ADOS* Autism Diagnostic Observation Schedule, *ADI-R* Autism Diagnostic Interview-Revised, *K-SADS-PL* Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime version, *INDT-ASD* INCLEN Diagnostic Tool for Autism Spectrum Disorders, *MCHAT* Modified Checklist for Autism in Toddlers, *ID* Intellectual disability

International Policy Context

Autism epidemiology has been an important tool for informing and advancing autism policy worldwide. Moreover, in recent years these advances have fallen at the intersection of relevant areas, broader than autism, including human rights policies for persons with disabilities, maternal and child health policies, and global mental health policies. Progress in reaffirming the rights of persons with disabilities has grounded and motivated policy development in other areas. The most widely used framework is the United Nations Convention of the Rights of Persons with Disabilities (UNCRPD; [3]), which outlines key principles, including respect for dignity, freedom of choice and independence, non-discrimination, full participation and inclusion in society, accepting people with disabilities as part of human diversity, equal opportunity, accessibility, and respect for the evolving capacity of children with disabilities and their right to preserve their identity.

The UNCRPD led to the development of a number of capacity-building frameworks, one of which is the International Classification of Functioning (ICF; [5]). Historically, autism research and care have relied of clinical definitions, such as those outlined in the International Classification of Diseases (ICD; [4]). The use of common diagnostic criteria is essential for comparable epidemiological estimates by defining "caseness." Change in these diagnostic criteria over time is one of the key reasons for the global increase in autism prevalence. Yet, major knowledge gaps remain in describing the functional impact of autism, which varies substantially across individuals. The ICF was developed to enable universal measurement of health and disability at both individual and population levels.

The ICF incorporates a description of various environmental factors in which function and disability occur. The framework has led to a number of instruments for assessing health status and disability across different cultures and settings [12–14]. The ICF Core Sets for Autism Spectrum Disorder were developed as a short list of domains most frequently impacted by autism (e.g., interpersonal interactions, emotional functions, and attention functions) [15]. These developments offer hope for improving clinical characterization in the context of epidemiological studies by offering comparable measures that cut across various forms of physical and mental disability. Currently, inadequate estimates of functional impact are available from epidemiological studies, which mainly rely on whether or not there is comorbid intellectual disability (ID; [16]).

Global advocacy and mobilization have also called for a move from awareness toward capacity-building in the area of autism [17]. In most world regions, autism awareness has advanced substantially but has not always led to a significant increase in available services. In recent years, the World Health Assembly adopted the World Health Organization's (WHO) Comprehensive Mental Health Action Plan 2013–2020 [18]. The plan supports access to "high-quality, culturally-appropriate health and social care in a timely way." The vision is a world in which mental health is valued and promoted, mental disorders are prevented, and persons affected by these disorders are able to exercise the full range of human rights and to access high-quality, culturally appropriate health and social care in a timely manner. The plan is guided by principles, such as universal health coverage, evidence-based practice, multisectoral approach, life-course approach, and empowerment of persons with mental disorders.

In response to the action plan, the WHO developed the Mental Health Gap Action Programme (mhGAP; [19]). It aims at scaling up care for priority mental, neurological, and substance use conditions particularly in poorly resourced settings, by delivering integrated packages of evidence-based interventions through collaborative networks of community-based services. It adopts a life-course approach and promotes the empowerment of users and families in decision-making and provision of care. The WHO has developed guidelines for assessment and management of priority mental, neurological, and substance use disorders by non-specialists in primary and secondary care levels [17]. Developmental disorders and other childhood mental disorders have been identified among key priority conditions to be addressed. In 2014, a specific resolution in respect to autism was passed for "Comprehensive and coordinated efforts for the management of autism spectrum disorders" [20]. As a result, a new intervention guide (The Caregiver Skills Training) was developed as part of the mhGAP to support and train caregivers and parents whose children are suspected or diagnosed with autism, and is currently being deployed across various world regions [21, 22].

Although autism is among a number of developmental disorders which falls within the umbrella of mental health frameworks, it also intersects with the area of maternal and child health. This includes the Global Strategy for Women's, Children's, and Adolescents' Health (2015–2030), a roadmap created to ensure every newborn, mother, and child not only survives but also thrives [23]. The roadmap is being implemented in part through the nurturing care framework [6]. The framework draws attention to the importance of early identification and intervention for children diagnosed with or suspected of a developmental condition. To achieve this, the framework calls for the integration of child development monitoring into maternal and child health programs to identify the earliest signs and symptoms in children with developmental disorders and to offer caregiver-mediated interventions. It also encourages investments from governments in the early years of childhood as a means to increase health, productivity, and inclusion of those affected.

Alongside international policy frameworks, a number of countries have responded by developing and refining specific

policies for autism. There is diversity in responses to the needs of autistic people and their families. In some countries changes in legislation (e.g., the UK Autism Act (2009) and the US Combating Autism Act (2006)) have resulted in a far-reaching impact on awareness, health, and educational services. More recently, France has also launched a national strategy [24]. Because different regions within a country can vary substantially in their models for healthcare organization and delivery, a number of regional action plans have also been developed (e.g., the action plans developed for the province of Quebec in 2017 [25] and for Wales in 2008 [26]).

More recent policies or updates to existing policies have drawn attention to the importance of innovative ideas that can inspire new models of care to overcome current barriers, including long wait times for services and the limited availability of services for adults with autism [26]. The success of these policies is highly dependent on implementation factors and mechanisms, such as the willingness of service providers to act, availability of the necessary infrastructure supports, and accountability mechanisms [25].

Epidemiological Estimates

The rapid progress in awareness and policy response worldwide has gone hand in hand with an increasing number of epidemiological estimates worldwide. The two most common epidemiological estimates are the number of cases in the population who have the condition during a specific period of time (i.e., prevalence) and the rate of new cases of the condition (i.e., incidence). Prevalence indicates the number of cases being identified and labeled as having the condition, whereas incidence is relevant for understanding risk factors and their potential change over time. Considering the wide range of factors that impact autism identification, incidence has been very challenging to estimate. In contrast, there is a very large and growing literature on prevalence.

Based on approximately 70 estimates, the last systematic review of global prevalence conducted in 2012 found that Autism Spectrum Disorders affect 1–2% of children, with a consistently higher prevalence in boys [16]. There was substantial variability in estimates within and across geographic regions and some estimates were very limited or completely absent from many world regions: Eastern Europe and Africa. Moreover, estimates were mainly available for children, with very few studies including populations older than 18 years of age.

Since 2012, many more studies estimating autism prevalence have been conducted, including in previously underrepresented regions and with individuals across the lifespan. Table 13.1 provides examples for the most recent estimates representative of different world regions with prevalence estimates as high as 4% of the general population. As evident in Table 13.1, recent prevalence estimates remain highly variable, in part due to the variability in methodological features, including sample characteristics and the methods used for case finding and definition.

Although epidemiological studies have rarely used comparable variables to describe clinical presentation, frequently reported variables are age, sex, and to some extent the proportion of cases with ID. As shown in Table 13.1, males consistently outnumbered females but with variable ratios across samples. Similarly, almost all studies report a subgroup with ID (or possible ID) with a proportion of up to 70%.

Prevalence estimates have also been used to develop indicators of the condition's economic and social impact. This includes the economic burden: costs of both direct (e.g., healthcare) and indirect costs (e.g., lost employment/ productivity) [27]. Estimates of the economic burden of autism range from 0.92 to 1.5 million £, 1.4 to 2.4 million USD, and 5.5 million CAD in the UK, USA, and Canada, respectively. Another indicator of health burden is estimated in terms of life years lost due to disability or disability-adjusted life years (DALYs; [28]).

In Europe, the Autism Spectrum Disorders in the European Union project (ASDEU) used comparable methodology to estimate and contrast cost across different European countries. The study revealed a substantial economic burden associated with autism but with substantial variability in individual costs between different European countries [29]. Similar findings were reported in similar studies worldwide, mainly in the US, UK, and in Canada [30–32]. Costs included healthcare, therapeutic, (special) education, and accommodation for affected individuals, as well as loss of lost productivity for the affected individuals and their family members.

Interestingly, in the US, healthcare costs only constituted a modest part of the total lifetime financial burden for children with autism, potentially reflecting varying degrees of access to healthcare programs [33]. Moreover, studies found that costs were correlated with functional impact of the condition; costs for those who are more severely impacted would include special education and/or residential care [32].

Taken together, epidemiological studies (Table 13.1) in autism have increasingly demonstrated the relatively high prevalence of the condition worldwide and quantified the health and economic burden in some countries. However, it is clear that prevalence estimates have been a moving target over time and there is no precise global estimate that reflects the worldwide situation. In fact, the substantial variability observed in the estimates can be, in part, accounted for by methodological and contextual differences among studies. These differences include general issues related to sample characteristics, such as sample size and representativeness that impact certainty in the derived estimates. Epidemiological studies rely on a variety of study designs with distinct case

finding and evaluation procedures, each with strengths and limitations. Methodological issues also interact with the community context where community awareness and capacity in health and education systems significantly impact autism identification, evaluation, and therefore prevalence estimates. Moreover, the clinical definitions of autism and its differentiation from overlapping conditions have evolved over time, impacting prevalence estimates. These considerations are discussed next as they relate to case finding, definition, and evaluation.

Case Definition

Two main clinical references most frequently used to determine autism "caseness" are as follows: The Diagnostic and Statistical Manual (DSM) of the American Psychiatric Association and the International Classification of Diseases (ICD) of the WHO, where the most recent editions are the ICD-11 [34] and the DSM-5 [35]. The DSM-5 has been in use in clinical settings in recent years, whereas the ICD-11 will come into use in 2022. Another reference used in a few epidemiological studies is the Chinese Classification of Mental Disorders (CCMD-3; [36]), although the ICD has also been in use in China as well. However, the CCMD itself was originally developed based on the DSM and ICD [37, 38], and therefore, a major impact on prevalence estimates is not expected.

Over time, changes in definitions of autism have influenced prevalence estimates. Starting with the narrowly defined Kanner's autism (1943), definitions progressively broadened in the criteria proposed by Rutter (1970) and became even broader over time in the two major nosographies used worldwide, the ICD and DSM. The earliest prevalence estimates based on older diagnostic criteria tended to reflect the more qualitatively severe forms of the phenotype of autism, usually associated with severe delays in language and cognitive skills.

The broadening of diagnostic boundaries to include milder forms has led to an increase in prevalence over time. Until recently, different prevalence estimates were derived for what were thought to be distinct diagnostic categories (or "subtypes") within a broader class of autism spectrum disorders (ASD) or "Pervasive developmental disorders" (PDD as an equivalent to ASD) in some nosographies. For example, the last systematic review in 2012 distinguished estimates for more narrowly defined Autistic Disorder from ASD, where the median was 17/10,000 for the former and 62 /10,000 for the latter [16].

The increasing refinement of the diagnostic criteria of autism over time has also resulted in the re-labeling of cases who would otherwise receive other diagnoses, a pattern known as diagnostic substitution [39–41]. Yet, not all studies have found direct evidence for diagnostic substitution over time [42–44].

The most recent versions of diagnostic classifications have generally drawn attention away from "sub-types" of autism and more toward the "spectrum." The DSM-5 features a single continuous spectrum (ASD), reflecting the variability of symptoms and the way they are expressed. Similarly, ICD-11 will not differentiate autism with and without intellectual disability. The impact of the most recent updates on prevalence has been investigated in a few studies, with inconsistent findings to date. Some studies found that the use of the DSM-5 criteria resulted in fewer diagnoses compared to the DSM-IV [45–48], whereas another study found that the DSM-IV PDD criteria and the DSM-5 criteria resulted in similar rates of diagnosis [49]. Considering the recency of the DSM-5 use in clinical settings, further research is needed to resolve inconsistent findings.

Another recent trend in diagnosis is the expansion of related conditions to include the "Broader Autism Phenotype" (BAP), a pattern of mild sub-clinical symptoms consistently observed in biological relatives of people with autism. A recent study compared prevalence of the BAP vs. autism over a ten-year period and found relative stability of the BAP but a significant increase in autism diagnosis [50].

In sum, the definition of autism has not been a static concept, and as a result, prevalence estimates reflect the evolution of clinical definitions over time. The most recent ICD and DSM updates have not yet been largely used in prevalence studies. Available estimates, such as those in Table 13.1, have used the older criteria or a combination of older and more recent criteria, reflecting the state of clinical practice when the data were collected. Notably, the increase in prevalence over time reflects the broadening of diagnostic criteria and the increasing reliance on a dimensionalization of the autism phenotype, blurring boundaries between autism, overlapping conditions, and typical development.

Case Finding

A wide range of study designs have been used to estimate prevalence with different approaches to case finding procedures. The largest epidemiological studies have been conducted through national surveillance programs. The most well established is the Center for Disease Control (CDC) surveillance system implemented through the Autism and Developmental Disabilities Monitoring Network (ADDM) [51]. The surveillance program has regularly published updated prevalence estimates of autism among eight-year-old children, and more recently among four-year-olds, who live in eleven sites.

More recently, Canada has also established the National Autism Spectrum Disorder Surveillance System (NASS) and

reported prevalence among 5–17-year-old children across six provinces and the Yukon territory [52]. The surveillance system identified children from health and social services or from educational records, depending on the data available from differences sites. Moreover, in one province, the data were extracted from a broader surveillance systems established for several health conditions, namely, the Quebec Integrated Chronic Disease Surveillance System [53].

In Europe, the availability of national health registries data has also allowed estimation of ASD prevalence in France, Denmark, Finland, and Iceland [54–56]. Such registries rely on substantial data available across the lifespan available through universal health systems, and therefore highly representative of local populations. Studies in other countries have also used administrative databases available through governmental hospitals [57], insurance providers [58], or educational systems [53, 59].

Other prevalence estimates were obtained from longitudinal cohort studies, such as the Neurodevelopmental Disorders Epidemiological Research in Spain [60], and the Longitudinal Study of Australian Children [61–63]. Similarly, national health surveys have also been used to estimate autism prevalence, such as the US National Survey of Children's Health [64] and the US National Health Interview Survey [65]. Finally, population-based epidemiological surveys have relied on a multi-stage approach: the screening stage casts a wide net to identify possible cases, whereas the final stage determines the proportion of screened cases who have a confirmed diagnosis [66–68].

Generally speaking, while surveillance systems, registries, and other administrative data may offer large sample sizes, true prevalence may still be under-estimated if there are many cases of autism in the community who do not have a diagnosis. These studies focus on populations who have access to services rather than sampling from the population at large, leading to possible under-estimation of prevalence. In contrast, population-based surveys that rely on active case finding procedures usually yield higher prevalence estimates than studies using administrative data [69]. However, the success of active screening procedures in identifying cases may be limited by other factors, such as population coverage, sample representativeness, and response rates from participants during different stages. Across all study types, other factors that influence prevalence estimates relate to the specific procedures used to evaluate cases, resulting in possible cases or confirmed cases of autism.

Case Identification and Evaluation

Different studies have to rely on different procedures to identify probable cases and to confirm diagnosed cases depending on the study design and the sample size of the population surveyed. Studies using administrative databases report cases as they have been identified in health or educational systems. Therefore, these studies are limited by the substantial variability in clinical practice and high potential for inconsistency across studies. For these reasons, the CDC established a more refined case evaluation strategy for their surveillance systems. In the first stage, records are screened using multiple linked data sources (health and educational), followed by a systematic review and scoring system for the data gathered in the screening phase combined with, in the less obvious cases, input from experienced clinicians, to ascertain cases of autism.

Population-based studies usually evaluate smaller samples and therefore use a multi-stage approach to screen the target population then confirm the accuracy of their final caseness. When the screening phase is completed, cases identified as positive screens go through the next step involving a more in-depth diagnostic evaluation to confirm their case status, either on the full sample or a randomly selected subsample.

Standardized tools frequently used during the screening stage of epidemiological studies include original or translated versions of the Modified Checklist for Autism in Toddlers (M-CHAT [70, 71]), Social Communication Questionnaire (SCQ [72, 73]), and the Childhood Autism Spectrum Test (CAST [60, 68]). When participants are directly examined for diagnostic confirmation, various approaches are used ranging from an unstructured examination by a clinical expert to the use of batteries of standardized measures by trained research staff. Standardized diagnostic tools that have been used to confirm caseness include the Autism Diagnostic Observation Schedule (ADOS [67, 68]), the Autism Diagnostic Interview (ADI [60, 68]), and/or other assessments used to characterize the heterogeneous profiles within the spectrum. Some studies have also used quantitative trait measures of ASD such as the Social Responsiveness Scale (SRS; [67]), and the Autism Quotient (AQ; [74]).

Choices made by different investigators regarding which tools to use for diagnosis are dependent on existing clinical and/or research capacity in the target community. Indeed, some epidemiological studies, such as those conducted in India [9, 75], have gone beyond raising awareness among policymakers about early identification and have also generated newly validated culturally appropriate screening and/or diagnostic tools, and supported training for clinical and/or research teams. As the number of these studies increases, there is also more awareness of the significant barriers simultaneously impacting research and clinical care in under-represented world regions, including the high costs and high level of specialized autism expertise needed for rigorous and standardized methods for case identification and evaluation [76–78].

For these reasons epidemiological studies have responded to the lack of contextually appropriate tools using different approaches. One approach that offers comparable estimates

across different world regions involves translations and/or cross-cultural adaptations of screening and diagnostic tools from their original form in English to other languages and cultural settings. One such example is the Arabic version of the M-CHAT [70]. Other studies have used original validated screening or diagnostic tools purposefully developed for a specific country/region, albeit limiting comparability with other tools used in autism research [9, 75, 79]. Other studies have used unvalidated although useful tools for their context. For example, a number of studies done in a school setting used a Teacher Nomination Form, where teachers are asked to identify possible cases based on non-technical descriptions of possible risk signs or symptoms [72].

Even when valid and reliable tools are used, the sensitivity of the screening methodology is difficult to gauge in autism surveys, because most studies do not report false negatives, i.e., true cases that are not identified in the screening stage. This implies that in general available prevalence of autism is under-estimated relative to true rates. Recent epidemiological studies in previously under-represented world regions have also been limited by relatively low participation rates and poor specificity of the screening tools used in the context of these studies [80].

In relation to case confirmation, when no standardized tools were available, investigators confirmed cases based on clinical judgement [81]. A few studies unfortunately omitted the case confirmation stage all together due to limited research or clinical capacity but acknowledged this as a methodological weakness and thus limiting the value of the resulting estimates [70, 82, 83].

Therefore, available studies highlight an unfortunate trade-off between methodological rigor vs. representativeness of samples in epidemiological estimates. The use of standardized tools increases comparability of findings. In the context of screening, knowledge of the sensitivity and specificity of the tools used is useful in understanding and interpreting resulting estimates. Similarly, the use of standardized tools for diagnostic confirmation reduces reliance on clinical judgment so that cases identified are comparable across studies. However, higher costs associated with the use of standardized tools have made research samples less representative of the world's population. This is especially the case in low- and middle-income countries where clinical and research capacity for autism are still developing.

Cultural Considerations in Case Definition and Evaluation

As the number of studies in diverse world regions has increased, there is rising debate over the universality of screening and diagnostic tools across cultures. Some argue that autism symptoms are consistent across cultures given the similarity in the neurobiology of ASD [84, 85], while others argue that cultural differences in social behavior can impact the perception of symptoms and the overall diagnostic process [86–88].

Some studies have directly examined cross-cultural performance of some of the tools used in epidemiological surveys. For example, a revised version of the M-CHAT (M-CHAT-R), a commonly used screening tool, across several countries was found to perform comparably in urban Turkey as in the US [89]. This finding is in line a number of other studies validating the M-CHAT for use across different cultures [89–92]. A more detailed examination of the tool has shown its overall good validity and reliability to detect ASD in Japanese 18-month-olds [93]. However, the same study also found that one of six critical items on the original M-CHAT, interest in other children, was less sensitive in detecting ASD cases, possibly because caregivers interpret lack of interest in peers as modesty or shyness rather than a symptom [93].

In relation to diagnostic instruments, an epidemiological survey in South Korea incorporated translation of diagnostic tools (ADOS and ADI-R) and concluded minimal impact of cultural variation when the assessments were conducted by experienced clinicians [85]. Other studies compared the AQ, a quantitative measure of autistic traits across different countries. One study using the AQ in a general population sample of university students in India, Malaysia, and then in the UK reported overall higher scores among Indian and Malaysian students compared to UK students [94]. Another study using the AQ as a caregiver-report in India, Japan, and the UK found strong overlap across the samples in the items that are most strongly associated with an autism diagnosis [95]. In contrast, other trait items indicated potential cultural differences. For example, an item on "enjoying doing things spontaneously" had excellent discriminant properties in the UK sample, but not in the Indian and Japanese samples, whereas the reverse was the case for another item on "enjoying social occasions."

Therefore, to the extent that evidence is available, cultural differences do not appear to limit utility of standardized screening or diagnostic tools. The variability in frequency of various items is not surprising since many different types of social/communicative deficits, including those that vary across cultures, can map onto the diagnostic criteria as defined by the DSM or ICD. What matters is that there are observable deficits, defined in whatever the culturally relevant form might be. Therefore, cross-cultural adaptation, rather than strict translation, would appropriately place emphasis away from specific behaviors that could potentially be more prone to cultural influence and toward the target underlying constructs. Similarly, cultural competency in the application of such standardized tools necessitates flexibility

as to differentiate between biologically driven symptomatology, the behaviors through which they manifest, and the expectations and perceptions of raters, including caregivers and clinicians.

Risk Factors Vs. Social Determinants

Although epidemiological estimates have primarily been used to inform policy and public health response, a number of hypotheses have emerged linking variations in prevalence in different populations and the time trends of increasing prevalence to possible differences in underlying risk factors. Once blamed on poor parenting, a biological framework to explain etiology of autism has been adopted. Initially, scientists searched for a single cause: the underlying gene or brain region that can explain all or most cases of autism. The search for underlying causes substantially advanced discovery of risk factors, but without producing "litmus tests" that can identify the condition at any point in development [96].

A more complex model of etiology is now adopted to map between risk factors and developmental outcomes. Interactions between various factors (biological, environmental, or social) further contribute to autism risk [97, 98]. The same risk factors that increase susceptibility for autism also increase risk for a wider range of neurodevelopmental disorders. Some of these conditions emerge early on (e.g., autism, ADHD, and language disorders), while others emerge later in life (e.g., schizophrenia and depression) [2, 96].

These risk factors modify brain development very early on in life resulting in the reorganization of neural networks that underlie cognition and behavior [96]. The resulting variation across individuals can be captured across multiple behavioral dimensions that evolve over time. Therefore, the impact of autism varies across the lifespan; some individuals with the diagnosis can lead independent and fulfilling lives, but many develop substantial medical, educational, and social difficulties that have a serious negative effect on their quality of life throughout their lifespan.

There is also speculation around a range of sociodemographic factors as potential risk factors for autism, including geography, ethnicity/race, nativity, and socioeconomic status [97, 99]. Variation in prevalence estimates linked to these factors has often been interpreted as reflecting differences in susceptibility to underlying genetic and/or environmental risk. Competing hypotheses use a social determinants perspective interpreting variation in estimates as reflection of health disparities resulting from differences in help-seeking and access to care rather than underlying biological or environmental risk. Available evidence linking epidemiological estimates to sociodemographic factors is discussed next.

Sex

Male sex is one of the most well-established risk factors for autism, giving rise to the notion of a "female protective effect," where females would require greater etiologic load to manifest the same degree of impairment as males [2, 96]. This pattern is confirmed by aforementioned reviewed epidemiological estimates, where males consistently outnumber females across studies. Paradoxically, the female protective effect also implies that when identified as having autism, females are more likely to exhibit a more severe phenotype. Consistently, most of the studies that reported IQ levels also found a higher proportion of autism cases with ID among girls than boys [54, 56, 100]. Similarly, another set of studies found that males with ASD were overrepresented in the range of higher IQ [74, 101]. These findings are consistent with previous observations in the US stating that the male-to-female ratio increased as the severity of ID decreased [102].

Complicating inference about sex as a biological risk factor are hypotheses about differences in ascertainment that may also account in part for the very wide range in male-to-female ratios. Some studies attributed sex differences to "boy-centric" aspects of diagnosis, such as girls having more socially appropriate restricted interests than boys, or overall higher levels of social skills, or lower IQ [70, 103]. Moreover, current assessment practices for autism are not optimized for girls relative to boys [104, 105]. In particular, the rate of false positives as a result of screening is higher for girls than boys [60, 105]. Therefore, while male sex can reasonably be used as a biological risk indicator, the impact of ascertainment issues on prevalence estimates is unknown.

Demographics

The last systematic review of global autism epidemiology [16] assessed the possibility of variation of prevalence by world region. A major challenge to addressing this question was that the majority of studies were conducted in the US and Northern Europe. Also, available estimates are not necessarily comparable because of methodological differences. Furthermore, several of the recently published studies in under-represented regions tended to have relatively small sample sizes and some of the studies did not include a stage of diagnostic confirmation [79, 82]. Therefore, while there are currently no sufficient studies with comparable methodology to address the question of regional variation, existing estimates show equivalent or greater intra- than inter-regional variability.

In contrast, some epidemiological studies have used comparable methodology to examining variation of prevalence among neighboring geographical regions. The largest of

these are studies conducted by the CDC in the US. The 2019 survey [106] focused on four-year-old children across seven sites found a threefold variation of rate by site, where Missouri had the lowest rates (85, 81, and 96 per 10,000 for 2010, 2012, and 2014, respectively) and the highest were in New Jersey (197, 221, and 284 per 10,000, for the same years, respectively). The latest survey among children aged 8 years and across eleven sites found a similar variation of prevalence by site, ranging from 131/10,000 in Colorado to 314/10,000 in New Jersey [51]. Across these studies, prevalence estimates were consistently higher in sites that reviewed education and health sources to identify cases compared with sites that relied solely on education records [106].

Another study from India estimated prevalence across five regions using comparable methodology. Among 2–6-year-olds, estimates ranged from 0.5% in North Goa to 1.7% in Palwal. Among 6–9-year-olds estimates ranged from 0.2% in North Goa to 2.1% in Hyderabad [75]. The authors attributed the variation to the potential variation in risk factors. However, pooled estimates across all sites revealed no differences in religion and/or rural vs. urban settings. Lai et al. specifically explored variation in rural vs. urban settings in Taiwan over time in the period from 2004 to 2010 [107]. They found overall a higher prevalence in urban vs. rural areas, but these differences decreased over time. Conversely, in a cross-sectional study conducted across different regions in India, autism prevalence was higher in rural areas relative to urban and tribal areas [9]. Taken together, there is no consistent sociodemographic factor that clearly accounts for the observed variation, but some findings may be related to regional differences in availability and/or accessibility of services.

Socioeconomic Status

An association between Socioeconomic Status (SES) and autism prevalence has been observed but not consistently across studies. For example, a positive association was found between SES and autism prevalence in the US [97], but not in other countries with universal health coverage, such as Sweden [108] and France (Malika [109]). Therefore, the observed association likely reflects inequalities in referral and/or access to services. However, the association may reflect and/or interact with other health, ethnic, and demographic disparities. For example, in the US, prevalence ratios for White relative to Black children were diminished after stratification by SES [97]. Similarly, in India, children from higher SES groups were more likely to have an autism diagnosis compared to lower SES groups, albeit without reaching statistical significance [9]. Paradoxically, prevalence of other childhood disabilities, including ID, is consistently higher among lower SES groups [110]. Taken together, the findings suggest that SES may not only be associated with other risk factors but can also modify outcomes.

Race/Ethnicity and Nativity

In past reviews of global epidemiology contrasting different world regions, no direct evidence for reliable differences worldwide based on race/ethnicity [16]. Racial disparities reflected in autism prevalence have been monitored in the US over time and the pattern of change suggests a "catch up" in diagnosis in minorities who were initially underdiagnosed [51, 106]. For example, in 2016, overall prevalence estimates were almost identical for White, non-Hispanic, Black, and Asian/Pacific Islander children, but lower for Hispanic children. Furthermore, the differences in prevalence between Whites and Black children were only observed in two sites [51]. Similarly, available data from insurance (Medicaid) shows higher prevalence among White as compared to Black, Latino or Hispanic adults [58]. Racial differences also impact clinical presentation in the US; a higher proportion of Black children with autism were classified in the range of ID compared with Hispanic and White children [101].

Outside of the US, a recent study found significantly lower rates of ASD among Arabs and Ultra-Orthodox Jews relative to the general population in Israel [111]. Other studies across different countries examined maternal nativity as a possible risk factor but documented both higher and lower prevalence in the offspring of immigrant mothers [112].

In Western Australia, one study reported administrative prevalence of ASD with ID while examining the combined effects of differences by maternal race/ethnicity, immigration status, and birth region [113]. Findings suggest that children born to Indigenous women and Asian immigrant women were much less likely to have ASD with ID than non-immigrant Caucasian women. In contrast, children born to Black women from East Africa were more likely than non-immigrant Caucasian to have ASD with ID.

Taken together, the evidence does not support the notion that race/ethnicity or nativity is a biological risk factor. Instead, disparities affecting these groups are reflected in autism prevalence, possibly in interaction with SES, by modifying patterns of help-seeking and access to care. Although available findings are limited and complex to interpret, they do raise the possibility that such racial/ethnic disparities may in some cases also modify developmental outcomes, resulting in lower outcomes for certain marginalized groups.

Time Trends

There is consistent evidence that supports a worldwide increase in prevalence estimates over time since the publication of the first epidemiological survey for autism in 1966 [16]. Time trends have been closely monitored in the US where one of the latest CDC reports estimating prevalence in 2010, 2012, and 2014 found an increase over time in prevalence in New Jersey but estimates remained stable in Arizona and Missouri [106]. The study also found that the proportion of children with ASD and ID was also stable over time [106]. Another study of Medicaid program users reported an increase of prevalence among adults with autism between 2006 and 2008 [58]. Moreover, time trends have reflected disparities in access to services among some racial groups.

Reported prevalence over time has also been consistently reported in various other countries, including in South Korea from 2008 to 2015 [114] and in Taiwan every year in the period from 2004 to 2010 [107]. Cohort effects have also been observed in France where prevalence was higher among children born in 2003 relative to those born in 1997 (children born in 1997 vs those in 2003; [56]). Similarly in Australia, prevalence was higher for children born four years apart (1999/2000 vs. 2003/2004; [61–63]).

Most reasons for this increase are uncontroversial and have been reviewed, including the substantial increase in community awareness and in public health response globally, changes in case definition that have broadened of diagnostic boundaries over time, increased diagnosis of milder forms, and increase in identification of autism in previously underdiagnosed populations defined by sex, geography, race/ethnicity, or SES. Time trends in autism prevalence reflect the combined effects of these factors over time. Therefore, the association between increasing prevalence and changes in risk factors cannot be taken as causation. To date, there is weak or conflicting evidence for the hypothesis that time trends may reflect increased exposure to environmental risk factors [115] or increased patterns of migration [116].

Conclusion

In recent years, the world has witnessed tremendous and positive improvements in public awareness and public health response. Among the benefits are the significant improvements in identification that have resulted in higher prevalence estimates over time. This increase reflects improved awareness among families and health care providers, and increase in identification and treatment services of autism, and more empowerment for caregivers to seek help despite the stigma. Epidemiological estimates have been increasing worldwide and continuing to inform the public health response.

Substantial variability in estimates within and across geographic regions is in part accounted for by known methodological factors causing over- or under-estimation of prevalence. Surveillance systems, national registries, and other administrative databases offer larger and more representative samples relative to other study designs, but they are usually associated with lower sensitivity for case finding, especially in areas with limited availability and/or access to service. Active case finding procedures in cohort studies or population-based epidemiological surveys may result in more rigorous estimates, but their results are often confounded by multiple factors related to the selected strategy for case finding, evaluation, and confirmation. Moreover, the availability of valid and reliable standardized tools for the target population, and the sensitivity and specificity of each tool in detecting suspected or actual cases also contribute to the accuracy of estimates. Cultural differences may indirectly influence screening or diagnostic confirmation in ways that are yet to be more carefully investigated as standardized and comparable tools become accessible across cultures. Despite these methodological issues, epidemiological estimates are still the best indicator of real-world trends in diagnosis, including factors that can impact time trends and/or geographic variability.

In addition to informing public health response worldwide, epidemiological findings have also been used to infer possible risk factors underlying variation in prevalence linked with a range of biological, social, and economic factors. Biological sex is the only factor among several that can be considered a clear risk factor gleaned from consistently higher male-to-female ratios across studies. Several other factors likely exert an influence on prevalence rates through complex interactions with biological risk factors, race/ethnicity, nativity, and SES that modify patterns of help-seeking and access to care, which in turn impact prevalence as well as developmental outcomes. Time trends in autism prevalence also reflect the combined effects of multiple biological, non-biological, and social factors over time. In all cases, future studies in autism epidemiology can benefit from further refinement in hypotheses about the influence of various risk factors. The positive increase in the number of studies conducted in previously under-represented regions also offers a unique opportunity to advance scientific discovery of risk factors while simultaneously expanding community capacity for autism identification and support.

Multiple-Choice Questions (MCQs)

1. What is the definition of incidence?
   (a) The number of cases in the population who have the condition during a specific period of time.
   (b) The rate of new cases of the condition in the population.

(c) The number of suspected cases of the condition in the population.

Answer: b

2. Which of the following statements is false?
   (a) The prevalence of autism has increased over time.
   (b) The prevalence of autism is variable within and across geographic regions.
   (c) The prevalence of autism is higher in North America relative to Europe.

   Answer: c
3. What are the limitations of registry studies in estimating autism prevalence?
   (a) They do not have a sufficient sample size.
   (b) They do not reflect patterns of change over time.
   (c) They do not directly confirm diagnosis using comparable criteria.

   Answer: c
4. Which of the following factors has been reliably linked with differences in prevalence estimates.
   (a) Biological sex.
   (b) Ethnicity/race.
   (c) Family income.

   Answer: a

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An Approach to the Diagnosis of Autism Spectrum

14

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Learning Objectives

By the end of this chapter, the reader should be able to

1. identify the diagnostic criteria for ASD and have an approach to eliciting this information on history and observation/interaction.
2. understand the benefits of standardized and allied health assessments, including when to use these in a diagnostic assessments.
3. approach a diagnostic disclosure in a sensitive and responsive manner.
4. adapt a diagnostic assessment to incorporate relevant cultural and ethnic considerations.

Highlights

1. In order to make an ASD diagnosis, the clinician needs to have evidence of difficulties with social communication and of the presence of restricted and/or repetitive behaviors. This evidence should include both reports from the child's daily life as well as direct observations of the ASD features.
2. The diagnostic assessment for autism spectrum disorder should be adapted based on the needs of the child and family. This might include addition of standardized assessments and/or allied health assessments.
3. A trusting therapeutic relationship is an essential element of the diagnostic process that begins at the first meeting with the family.
4. A family's cultural and ethnic background informs when and how the child presents for a diagnostic assessment, the information received, the interaction with the child, and the family's understanding and perception of the diagnosis.

Introduction

The diagnostic assessment for autism spectrum disorder (ASD) can vary considerably based on the needs of the child being assessed and the needs of their family. The diagnostic criteria for ASD are detailed in the Diagnostic and Statistical Manual, 5th edition (DSM-5) [1] and include two main domains of impairment: social communication and restrictive/repetitive behaviors. In addition to fulfilling the necessary diagnostic criteria, the DSM-5 also sets out that the symptoms must be present early in life and cause clinically significant impairment. The impairment should not be better explained by intellectual disability or global developmental delay, although these conditions frequently co-occur with ASD. The clinician should specify whether the child also has intellectual impairment, language impairment, medical/genetic conditions, relevant environmental exposures, and other neurodevelopmental, mental health, or related disorders.

ASD assessment encompasses more than just making a categorical determination of whether the child has ASD. This is due to the numerous co-occurring conditions that frequently accompany ASD. This reality also helps emphasize an important point: ASD diagnostic assessment is best conceptualized as an ongoing process, stretching across geography, providers, and most importantly, time. As children grow

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and develop, their needs change and co-occurring conditions can emerge. It is important to ensure that access to ongoing assessment is available to continue to meet the medical, behavioral, mental health, and psychosocial needs of children with ASD and their families.

History Taking

In many cases, the diagnostic assessment begins with asking the family and child about possible symptoms of ASD as part of a developmental and medical history. There are some key considerations for the history that set the tone for the rest of the assessment and the family's trust in the validity of the results. Important first steps include determining the family's understanding of the reason for the assessment, including whether they know their child is being evaluated for ASD. It is important to inform the family about what will happen during the assessment, including who will do what elements of the assessment, when this will occur, and whether the child needs to be present for all aspects. Throughout, it is of utmost importance that the clinician listens to and validates the family's experiences.

The history must contain an elicitation of any signs and symptoms of ASD. In contrast to some other aspects of developmental histories, the history for ASD is often focused more on qualitative descriptions (i.e., how does the child indicate they want something) than quantitative milestones (i.e., did the child do this by this age). Table 14.1 contains a list of features and sample questions that can identify challenges in social communication and restrictive/repetitive behavior. One of the best tools at the clinician's disposal is asking for examples of a time when the child demonstrated the particular skill or behavior in question. In addition, clinicians can ask families to bring videos of the child which can be an excellent way to understand how the child interacts and communicates with others in their natural environment.

The history must also cover other developmental domains beyond those directly concerned with the ASD diagnostic criteria. These are important to determine the overall developmental level of the child, which is essential to set the expectations for the child's social communication skills and behaviors. For instance, if the child's developmental level is less than 12 months of age, they may not be expected to have developed skills of pretend play or joint attention. In addition, these developmental domains are critically important to explore due to the high rate of co-occurring conditions in ASD. Motor domains are important to identify possible developmental coordination disorder and other motor coordination issues, which are common in autistic children and youth [2]. Clinicians must inquire about the child's cognitive skills to screen for possible intellectual or learning disabilities, as outlined in the DSM-5. Many children with ASD have symptoms of attention-deficit/hyperactivity disorder (ADHD) [3]; here, it is important to consider the child's developmental level to gauge the expectations for the child's attention.

In addition to the developmental disorders, there are many mental health conditions that frequently co-occur with ASD, including anxiety in 20% and depression in 11% [4]. For children diagnosed in the toddler/preschool years, it is important for clinicians to continue to screen for mental health conditions, particularly in the adolescent and adult years. For children and youth who are in the school age and adolescent years at the time of diagnosis, these conditions may already be present and may make the diagnostic assessment more complex. In these cases, it can be helpful to pair descriptions of current challenges with accounts of what the child was like in their early childhood years.

There are many medical comorbidities that frequently occur in autistic people. It is important to ask whether the child is taking any medications, vitamins, supplements, or other natural health products, as well as inquiring about any allergies. Gastrointestinal issues are more than four times more likely to occur in children with ASD compared to typically developing children and can include constipation, diarrhea, gastroesophageal reflux, and others [5]. Related to this, many children with ASD have restrictive diets, and a good dietary history is important to screen for possible nutritional deficiencies. Sleep difficulties frequently occur in children with ASD [4, 6], including delayed sleep onset, frequent night-time awakenings, parasomnias, and sleep apnea. Again, a careful sleep history is important, including total duration of sleep in a 24-hour period, night-time routines, what happens when the child wakes up at night, and any snoring or pauses in breathing. If the child is getting enough sleep, it is important to ask whether any aberrant sleep behaviors are seen as problematic by the family, as this may not be a priority for that family at that time.

The family and social history provide a great deal of important information. A thorough history of the first-degree relatives is necessary, and should include their ages, any health conditions, any learning/developmental diagnoses, mental health conditions, education, and occupation. It is also important to inquire about any pregnancy losses for the parents or early childhood deaths in other relatives. The clinician should also inquire about learning/developmental issues in the broader extended family. From the social perspective, the clinician should directly inquire about who the child lives with, including careful delineation of this for shared custody situations. When the child's parents are separated or divorced, the clinician should obtain details about the custody agreement if available, including who has access to clinical information. This is particularly important if only one parent is present at the assessment. If there is any history of abuse or neglect, the clinician should obtain details about

Table 14.1 ASD characteristics and strategies for history and observation/interaction

ASD Feature	Questions to ask on history	How to elicit on observation/interaction
Social communication and interaction		
Showing or giving toys	Does your child ever show you toys they are playing with? Does your child give you toys because they are interesting (instead of just for help)? Does your child share their toys with other children to start a play activity?	Does the child show toys to their family members by orienting it toward them? If so, do they do this with coordinated eye contact? Does the child share toys with their parents out of interest, instead of just doing this to ask for help?
Initiating and responding to joint attention	Does your child try to draw your attention to something interesting (i.e., 'look mommy, an airplane!'). If so, do they look at your eyes when they do this? Can you draw your child's attention to something interesting (i.e., 'look at that!')?	Does the child point to something they cannot reach, such as a poster on the wall, and pair this with eye contact with another person? Once you have the child's attention, point to something that none of you is touching and say 'look!'
Response to name	Does the child look up when you call their name?	Call the child's name and observe whether they look at you. If they do not respond, ask a familiar person to call their name.
Comforting and sharing emotions	How does your child share their feelings with you? What does your child do when someone is upset or crying?	Smile at the child and see if they smile back. If they do not, have a familiar person smile at the child and watch to see if they smile back.
Conversation	What is it like to have a conversation with your child? Is it one sided or does it go back and forth? Do they tend to take the conversation in their own direction?	Make a comment (i.e., 'I saw something neat yesterday') and see if the child asks follow-up questions. If not, ask the child a direct question. Observe if the child stays on topic or takes the conversation toward a preferred topic.
Eye contact	Have you had any concerns about your child's eye contact? Do they look in your eyes when they are talking to you or asking for help?	Observe when the child makes eye contact. Important times to observe include when the child is asking for something and when someone else is trying to get their attention.
Gestures (including pointing)	Does your child point to things? Does your child use gestures such as nodding or shaking their head, or waving? Does your child describe things with their gestures, such as showing you that something is big?	Observe whether the child points. Do they do this just to request, or also to share interest? Is their pointing coordinated with eye contact? Does the child use other gestures, such as conventional gestures (nodding/shaking head) or descriptive gestures? Note unusual physical forms of communication, such as pulling a parent by the hand without eye contact or using someone's hand as a tool.
Facial expressions	Can you tell how the child feels by the look on their face? Do they show you their face to let you know how they are feeling?	Observe the child's facial expressions, as well as whether these are directed to others and paired with eye contact.
Interaction with caregivers	What does your child like to do with you? What do they like to do on their own? Do they tend to play more on their own or with another person? How does your child indicate that they want you to play with them? What does your child do if you try to join their play?	Observe whether the child shares toys, shows toys, or other ways they get you or their caregiver to play with them. How does the child respond if you try to play with them? Is it possible to have a sustained back-and-forth play with the child?
Interaction with peers	Is your child interested in other children? Do they approach other children to play? How do they do this? How does your child respond when other children approach them? Does your child have a preferred playmate or friend at school/child care?	Observe how the child interacts with their siblings or with other children in the waiting room.
Interactive pretend play	Does your child do imaginative play, such as feeding a stuffed animal or making toys talk to each other? Do they do any role play, such as pretending to be superheroes? Do they involve you or others in this play?	Bring out toys that are suited for pretend play, such as a doll/action figure, pretend food and dishes. Encourage the child to join you in play. See how they participate. Try to add a new element to the play and see how they respond.
Restricted or repetitive behaviors or interests		
Stereotyped motor movements	When very excited or upset, does your child ever tense up their body or flap their hands? Does your child walk on their toes?	Observe how the child moves their body, particularly when excited. Relevant movements can include tensing of the body, flapping hands, posturing fingers, and walking on toes.
Repetitive/stereotyped play	Does your child have any unusual or repetitive ways of playing with toys? Do they line up, sort, or spin toys?	Observe whether the child plays with their toys in their intended fashion, or if they line up, sort/organize, spin, or have other repetitive forms of play.

(continued)

Table 14.1 (continued)

ASD Feature	Questions to ask on history	How to elicit on observation/interaction
Repetitive/ stereotyped speech	Does your child ever repeat what other people say in exactly the same way (echolalia)? Does your child ever repeat things from television, videos, or books? Does your child mix up "I" and "you," such as saying "you want a cookie" instead of "I want a cookie"? Does your child have any words that are unique to them that others do not understand (neologisms)?	Listen to what the child says. Does the child echo what others say? Does anything the child says sound as though it is being repeated verbatim from another source? Does the child mix up pronouns, specifically "I" and "you"? Does the child use any unusual words?
Routines, rituals, and difficulty coping with changes	Is there anything your child needs to have done the same way, every time? How does your child cope if their schedule or routine changes? How does your child deal with changes, such as moving furniture to new places in the home?	Observe whether the child has ritualized behavior, such as needing to complete a list or saying things in order. Recite a list and playfully put an item out of order to see how the child reacts.
Transitions	How does your child do with changing from one activity to another? Do they have to complete something before they can move on?	Put away one toy/activity and take another to see how the child reacts.
Rigid behaviors	Is your child very strict with following certain rules, and enforcing these rules with others? Does your child understand jokes and sarcasm?	Play with a toy in an unexpected way and see how the child reacts. See how the child responds to jokes and non-literal phrases (i.e. hit the road).
Intense or unusual interests	Is there anything that your child is totally obsessed with? Does your child have any unusual interests?	Observe whether the child brings up particular topics of interest, or whether they have brought any special items with them to the visit.
Sensory aversions	Is your child bothered by loud noises, bright lights, certain fabrics or clothing tags, or strong smells? Does your child have difficulty with any food textures?	Observe any aversive reactions to the toys (such as toys that make noise) or environmental stimuli.
Sensory interests	Does your child inspect toys or other items very closely? Does your child like tight hugs or the feeling of pressure?	Note whether the child puts toys up to their eyes, rotates toys while looking at them, or pushes cars back and forth at eye level. Observe whether the child squeezes, sniffs, or licks/tastes toys (mouthing is developmentally appropriate before 2 years).

Note: The applicability of the various questions and prompts will depend on the child's developmental level. This list is not intended to be a comprehensive list or test of all features of ASD

the nature and timing of the harmful event(s) to screen for possible attachment disorders, whether child protective services were involved, whether the child was removed from the care of the biological caregiver, and who has decision-making power for the child at the present time. Finally, it is important to inquire about the family's broader support network, including who provides childcare, and if the family has a broader community of support.

Collateral Information

Observations of the child from other environments provide rich information to include in the diagnostic formulation. For younger children, child care staff have the opportunity to observe many social interactions between the child and their peers, which directly informs the ASD diagnostic criteria. For older children, teachers also have this opportunity to observe children interacting with their peers for many hours each day and can provide valuable information. Many clinicians obtain information from these individuals using questionnaires, which can provide an efficient means to solicit their input. In cases where more detailed information is required, spending the time to speak with child care staff or teachers allows for customized questions specific to the child and is a worthwhile investment. The child may have had assessments by other allied health professionals, such as speech-language pathologists or occupational therapists, which also provide complementary assessment information that can be incorporated into the formulation.

Observation and Interaction

The goal of the observation and interaction is to obtain first-hand evidence of the child's communication abilities, social skills, behaviors, and other developmental milestones. The observation of children with suspected ASD begins the moment the child is in view. There are many useful observations that occur outside of the designated observation and interaction time in the assessment. For instance, the clinician's waiting room is a space to see how the child interacts

with toys and potentially with other children in an unstructured setting. If the child is in the room during the history with the parents/caregivers, it is important to have toys available and to continue to observe how the child interacts with the toys and with the other people in the room. Finally, the clinician should ensure that there is a designated time for observation and interaction with the child to elicit signs and symptoms of ASD. Suggestions for eliciting observations associated with various symptom domains are provided in Table 14.1.

The clinician should be aware of logistical, environmental, and temperamental factors that may influence the quality and trustworthiness of the observation and interaction. The child may be bored or tired by the end of the history, and it may be necessary to bring them back at a different time to perform the observation and interaction. A key logistical consideration for very young children is whether they take a nap; care should be taken not to schedule the assessment during this time. An interpreter should be considered when the assessment is conducted in the child's non-dominant language, recognizing that this may impact the quality of the interaction with the child. The environment in which the assessment takes place can also affect the interaction. Many children may associate clinical environments with unpleasant events, such as receiving vaccinations, which may cause them to be anxious in similar environments. It is important to have appropriately sized furniture for children and to have items or pictures out of reach to facilitate initiation of joint attention. Finally, the temperament of the child may influence the interaction. Highly anxious children may not make many social overtures and may be difficult to engage. Here, it may be helpful to give them time to slowly warm up to the clinical environment before attempting to engage them. If available, observing the child and parent/caregiver behind a one-way mirror is a way to obtain observations of the child when they are more comfortable. Other children may demonstrate a high activity level and impulsivity, which may decrease the quality of their social overtures and responses. The child's overall temperament should be taken into consideration when interpreting the observations.

The clinician should observe and attempt to elicit signs and symptoms of ASD, being cognizant of what they do see (for instance, motor mannerisms, stereotyped play), and what they do not see (for instance, the child does not point or show toys to others). The latter takes conscious effort and periodic check-ins to make note of what the child is not doing. There are standardized, semi-structured interactions that can be administered and require training, such as the Autism Diagnostic Observation Schedule, second edition [7]. Even when using a standardized tool, there are some general principles to set the stage for a trustworthy interaction. First, it is important for the clinician to be a good playmate. This involves getting down to the child's level and adopting an animated persona that is responsive to the child's needs, for instance, taking quieter tones with shy or anxious children. Second, it is important to take notes throughout the assessment instead of relying on memory or gestalt. Finally, because the interaction often takes place in a clinical environment, it is important to check in with the child's family to ensure that the interaction reflects the child's typical behavior.

Standardized Testing

Many standardized tests have been developed to assist with ASD diagnosis, including those that help to elicit characteristics of ASD from families and semi-structured interactions that allow the clinician to directly observe these characteristics. It is important to note that no single test result is in itself diagnostic, and all test results must be integrated with the whole clinical picture to arrive at the diagnosis. There are many considerations when selecting a test. First, the clinician should be aware of the test's performance (sensitivity, specificity, positive and negative predictive values). There are often requirements that must be met in order to administer standardized testing that can include professional designations and tool-specific training, which can vary considerably in intensity. The clinician should also consider contextual factors related to the test, including performance in different genders and relevance to families and children from differing cultural backgrounds (see section on Cultural Considerations below). Finally, while standardized testing can contribute highly valuable information toward ASD diagnosis, care needs to be taken to ensure that a requirement for standardized testing does not delay access to ASD diagnosis.

Physical Examination

Due to the high number of co-occurring medical conditions in ASD, it is important for all newly diagnosed children to undergo a physical examination. Non-medical clinicians, such as psychologists, can still diagnose ASD when they feel the diagnostic criteria are met and can make a subsequent referral to a physician or nurse practitioner to complete the physical examination.

Wherever possible, the physical examination should occur after the interaction/observation with the child. A full general screening physical examination is preferred. Specific elements of interest include growth parameters (including head circumference), inspection for dysmorphic features, a skin examination (ideally with a Wood's lamp to look for signs of neurocutaneous syndromes such as neurofibromatosis or tuberous sclerosis) and a neurological examination.

Audiology assessment is outside of the scope of most physical examinations and the child should be referred for this. The child should also be referred for vision assessment as necessary.

Allied Health Assessments

Clinical guidelines often recommend the involvement of multidisciplinary teams in the diagnosis of ASD [8]. Members of the team may include a pediatrician or developmental paediatrician, psychologist, child psychiatrist, speech and language pathologist, and occupational therapist. Real-world practice patterns have been found to vary across Canada, the United States, and Australia and include both solo and multidisciplinary team assessments [9–11]. Research comparing the diagnostic accuracy of solo versus multidisciplinary team assessments is limited. Possible advantages of multidisciplinary team assessments include the identification of co-occurring or alternative diagnoses and support in informing treatment and intervention for the child [12]. On the other hand, one should consider the impact of these assessments on diagnostic wait times, because they require multiple team members. One solution may be to tailor the need for team members based on the complexity of the child's presentation, co-existing health or mental health concerns, and psychosocial history. More recent Canadian guidelines have outlined three different approaches to the diagnostic assessment which include solo assessment, a shared care model where a clinician collaborates with another health care provider, and a team-based interdisciplinary or multidisciplinary approach [13]. Ultimately, one should consider adapting the approach to the diagnostic assessment flexibly based on the needs of the child and family, given the heterogeneity in a child's presentation with ASD.

Diagnostic Feedback

Diagnostic feedback refers to the process of communicating a diagnosis of ASD to the family. This process is one that begins as early as the first interaction between the clinician and family when the topic of ASD is introduced. The first encounter is not only important for gathering information to inform the diagnostic assessment, but it also builds rapport and sets the tone for the assessment and feedback. Attending to the therapeutic relationship from the start will impact how a family perceives the assessment as a collaborative, individualized, family-centered process. During the early stages, it is important to ascertain the family's beliefs, values, and expectations as well as their level of understanding of ASD because this information will allow the clinician to better gauge the family's needs in a sensitive and responsive manner during feedback.

Care should be taken in setting the stage for the feedback session. The feedback should be conducted in a private space with minimal interruptions. Individuals present during the feedback should include the child's main caregiver(s) and/or legal guardian, the child or adolescent (if appropriate based on their developmental level) and the clinicians who were involved in the diagnostic assessment. An interpreter should be available to the family if needed. Timing of the feedback session is also important in that it should occur soon after the assessment is completed. In addition, sufficient time should be allotted for feedback so that the family's concerns may be adequately addressed. If the initial time allotted to the family is insufficient, the clinician should consider setting up another meeting with the family to address further concerns.

While clinicians may address feedback in a variety of ways, the content of the feedback should include a review of the parents' expectations and understanding of ASD, an assessment of the child's strengths and needs, a clearly stated diagnosis, a discussion about causes, future implications and prognosis, and provision of recommendations for the child. Families should be encouraged to share the results of the feedback with others such as teachers. Clinicians should express hope for the child to help families see the possibilities for their child and the options available to help their child learn new skills. Although this provides an overall framework for the feedback session, the clinician should be attuned to the family's needs and adapt flexibly to encourage a dialogue between the clinician and family.

The manner in which feedback is delivered is just as important as the content itself. The clinician should pay attention to the family's non-verbal communication as this may provide insight into the family's stage of acceptance of the diagnosis. Another skill that the clinician should cultivate is listening in a reflective and empathic manner to respond to the family's needs more effectively. At the conclusion of the visit, the family should be provided with written materials including the diagnostic report, recommendations, and further information as necessary so that the family may reference these materials at a later time. It is also helpful to provide details about who will be following the child, when this follow-up will occur, and what the family can do if they have questions that arise before the follow-up.

Family Preferences

A family's journey through the diagnostic process represents an emotionally intense experience that begins long before a child's diagnostic assessment. Parents themselves go through a highly individualized experience in coming to terms with

the possibility that their child may potentially have ASD [14]. Each caregiver may be at a different stage of emotional adaptation upon arriving at the diagnostic assessment, which may impact their level of motivation to receive a diagnosis. Unfortunately, their journey may be complicated by visiting multiple professionals and experiencing long waiting lists for the developmental assessment, which is associated with higher levels of stress and reduced satisfaction with the diagnostic process [15, 16]. The assessment process itself has been found to be lengthy and emotionally draining by caregivers. Therefore, informing families prior to the assessment about the duration, components/assessment tools, and professionals involved in the assessment is important. Clinicians should inform parents about the need for the assessment and that ASD is suspected prior to the assessment, which may help prepare parents for the assessment and later acceptance of the diagnosis if given.

Families have reported varying experiences with diagnostic feedback. Positive aspects of receiving a diagnosis can include receiving an explanation for a child's behavior, reduced guilt about a parent's caregiving approach, and the ability to obtain information and find supports for a child [17–19]. However, a growing number of studies have shown a high degree of parental dissatisfaction with the diagnostic experience, which is linked to higher levels of parental stress [16, 20]. Factors correlating with these impressions include the interpersonal skills of the clinician providing the diagnosis, such as honesty and empathy; the parent–clinician relationship, including ensuring that parents are considered equal partners in decision making; the clinician's effort at getting to know the child as a whole; and the clinician's ability to listen to the family, give them time to absorb information, and ask questions [19, 21, 22].

When parents arrive at feedback, a complex array of emotions may arise. This experience has been likened to the 'sounding of an alarm,' whereby parents experience shock at hearing the diagnosis and subsequently experience a 'swell of distress and uncertainty' [23]. A range of emotions has been described during this process, which encompass relief to despair and grief. Parents value having a clinician who is able to pause and listen to their reaction, validate and accept their feelings and experiences, and provide an optimistic view of their child's future. As a result, recognition of a parent's experience and checking in with parents during feedback about how they are feeling will help the clinician adjust their tone and approach to feedback in a supportive manner for the family.

Once a diagnosis is shared with the family, further considerations include the provision of information, resources, and support [22]. Parents appreciate being provided with information including written materials about ASD, intervention services, school support, alternative therapies, and ways to explain the diagnosis to their child. Recommendations should be tailored to the needs of the child and family. Both the volume of information and timing of information delivery should be considered thoughtfully by the clinician so as not to overwhelm the family. Since feedback is already an emotionally overwhelming experience for parents, providing a separate follow-up visit may be helpful to enable parents to formulate questions and better retain information. Parents juggle many responsibilities for their child which may include advocating for their child's needs, exploring available resources, managing their child's supports, and providing behavior interventions [14]. Service limitations, long waiting lists, and lack of centralized coordination of services may also lead to feelings of uncertainty and hopelessness. Therefore, professional support and direction provided by the clinician can help reduce this burden for parents.

Cultural Considerations

Culture and ethnicity are important considerations that influence the diagnostic process for families. Studies have shown that the age of first parental concern for ASD features is earlier in Western countries (age 14–19 months) compared to other countries (age 24–31 months) [24]. Obstacles that families may encounter in accessing a diagnosis include affordability, availability, geographic access to services, and language and literacy barriers [24]. Diagnostic delays also exist among immigrant families in Western countries, with children more likely to be diagnosed after the age of 4 years compared to non-immigrant children [25]. Systematic disparities in access to care play an important role in these delays; for instance, parents of Black autistic children report that their concerns were often ignored and that racial bias impacted the care they received [26].

It is important for clinicians to be aware of how cultural differences may impact the history they receive, their observations, and their own thinking on the likelihood of an ASD diagnosis. Reduced awareness or knowledge of ASD features and of a child's developmental trajectory in some cultures may contribute to diagnostic delays [27]. It is important to recognize that accepted social norms of behavior may vary across cultures, which may impact what falls within or outside of expected skills and behaviors [28]. While some features of ASD, such as reduced eye contact, may be regarded as atypical in Western countries, other cultures may consider this feature to be a social norm or put less weight into its significance [24]. Stigma experienced by the child and caregiver with the diagnosis may also prevent a family from accessing a diagnosis, as it may lead to feelings of shame, social exclusion, and judgment [27, 29]. A caregiver's perception of the clinician providing the diagnostic assessment may impact the acceptability of the diagnosis, as well as the caregiver's desire to pursue further ASD supports for their

child. Clinicians should be aware that distrust with the health care system exists in many cultures due to previous negative experiences of discriminatory treatment or biases of health care providers [24].

Given these considerations, emphasis should be placed on developing a culturally sensitive, family-centered approach to optimize a family's experience with the diagnostic assessment. Clinicians should explore a family's cultural beliefs and social context, which may influence perceptions of their child and their understanding of ASD. Respecting the values and dignity of the family while being aware of one's own biases will enable the clinician to develop a partnership with the family wherein expectations and goals are aligned [30]. It will also enhance the engagement of the family during diagnosis and for future intervention. Development of culturally appropriate, validated screening and diagnostic tools should be a priority for future research.

Multiple Choice Questions

1. The domains from the DSM-5 for ASD diagnosis are as follows:
   (a) Communication, social skills, and behaviors.
   (b) **Social communication and restricted/repetitive behaviors.**
   (c) Sensory differences, social challenges, stereotyped behaviors.
   (d) None of the above.
2. Which of the following is/are co-occurring conditions in ASD?
   (a) Depression.
   (b) Developmental coordination disorder.
   (c) Anxiety.
   (d) **All of the above.**
3. Why is it important to understand a child's overall development when making an ASD diagnosis?
   (a) You cannot make a diagnosis in a child with a developmental level less than two years of age.
   (b) The overall developmental level helps set expectations for skills in ASD-relevant domains.
   (c) Children with a developmental level less than 12 months would not be expected to have some of the skills commonly evaluated in an ASD diagnostic assessment (joint attention, pretend play).
   (d) **B and C.**
   (e) All of the above.
4. Cultural and ethnic considerations are important in which of the following parts of the diagnostic assessment:
   (a) Interpreting the child's developmental skills.
   (b) Selecting relevant assessment activities and understanding the child's performance.
   (c) Communicating the diagnosis to the family.
   (d) **All of the above.**

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Autism Spectrum Disorder: Cognition

15

Elizabeth Kelley and Alison Dodwell

Learning Objectives

1. Describe each theory of cognition in autism and explore why it is **not** seen as a definitive theory of autism.
2. Define top-down and bottom-up attentional guidance and identify how attentional systems are postulated to dysfunction in ASD based on experimental evidence.
3. Explore research surrounding hyper- and hyposensitivities to multiple domains of sensory perception, as well as atypicalities in self-perception and social perception associated with ASD.
4. Enumerate the percentage of individuals at each level of intellectual functioning in the study discussed.
5. Characterize the weaknesses of research in intelligence, academics, and general cognition.
6. Compare and contrast the research findings in semantic vs. episodic memory.
7. Support the statement that, 'Executive functioning is most affected in ecologically valid contexts.'
8. Summarize the strengths and weaknesses of the Theory of Mind theory using evidence from the social cognition section of the chapter.
9. Document which aspect of language is most affected in ASD and explore the potential reasons why.
10. Recommend three aspects of research in this field that need to change in future studies and justify your recommendations.

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Introduction

Autism Spectrum Disorder (ASD) is defined by social communicative difficulties as well as restricted and repetitive behaviors and interests [1]. The difficulties with social communication range from challenges in interactions with others to problems making friends; many of these specific difficulties will be discussed in this chapter as they relate to cognition. Restricted and repetitive behaviors and interests refer to individuals with ASD's narrow range of things that they like to do and that they are interested in, which will also be discussed here as they relate to cognition, albeit to a lesser degree. One of the critical points to keep in mind as you transverse this chapter is that autism is by nature a very heterogeneous disorder. Some individuals with ASD are completely mute, have very low IQ, spend a great deal of time engaging in non-meaningful self-stimulatory repetitive behaviors, and are placed in special education classrooms. Other individuals with ASD are fluently verbal, have average to high IQ, talk incessantly about their restricted interests, and are placed in mainstream classrooms without any extra help. It is well agreed upon in the field that while it is certainly a genetically based disorder (though behaviorally defined), there are many different causes of autism—this heterogeneity can make consistent and powerful effects of the disorder challenging to discover.

We will begin the chapter by discussing the four main theories put forward to account for the cognitive differences seen in individuals with ASD. We will then talk about attention in ASD, including social attention, followed by sensory perception and self and social perception. From there, we will move into intelligence, academics, and general cognition, followed by a discussion of memory. Executive functioning difficulties will be discussed, as well as problems in Theory of Mind and social cognition more broadly. We will then discuss the patterns of findings in the language field, breaking the field down into general language findings, phonology and syntax, semantics, and finally pragmatics. We

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will finish the chapter with some concluding thoughts about the limitations of the research, as well as potential future directions.

Theories of Autism Spectrum Disorder

When Kanner (1943) first described the disorder of autism, he focused on its biological nature, but he quickly jumped on the psychoanalytic bandwagon of the time and blamed these children's autistic behavior on the 'coldness' of the mother [2, 3]. By the 1960's and 1970's, autism was thought to be caused by faulty sensory input or a dense language disorder [2, 4]. Since the 1980s, four main theories have been put forth to explain the nature of autistic cognition: the Theory of Mind theory [5], the Weak Central Coherence theory [6], the Executive Dysfunction theory [7], and the Social Motivation theory [8].

Theory of Mind refers to the ability to understand that others have beliefs that differ from our own and additionally, and that they act on those beliefs [9]. In 1985, Baron-Cohen and his colleagues tested theory of mind abilities in children with ASD using the classic false-belief task, which measures a child's ability to understand that people will act on the knowledge that they have, even if that knowledge is incorrect [5]. The researchers found that 80% of children with ASD were unable to pass this task [5]. Baron-Cohen posited that this was due to a domain-specific module that was specially impaired as he noted that some other social tasks were intact [10]. As will be described later in the chapter, many children with ASD can pass basic theory of mind tasks, thus, leading researchers to speculate that theory of mind is impaired in some children, but it is not domain specific or a universal difficulty [11]. Another problem with the Theory of Mind theory is that it does not explain the restricted and repetitive behaviors that are core symptoms of ASD [6]. Furthermore, if theory of mind was the primary cause of ASD, it should correlate with a measure of autistic severity. Joseph and Tager-Flusberg (2004) found no association between theory of mind and autism severity [12], although more recent studies have found this correlation [13].

The Weak Central Coherence Theory posited that individuals with ASD are very good at perceiving the details of a situation but are unable to align those details or make inferences in order to come up with the gist of the situation (i.e., the 'big picture') [6, 14]. Support for this theory arose from research showing that individuals with ASD demonstrated superior performance on the Block Design measure of the Wechsler scales, and the Embedded Figures Task (see Fig. 15.1) [6]. However, further research demonstrated mixed findings on these tasks, as well as other tasks which require a focus at the local, rather than the global level, such as the Navon task (see Fig. 15.2), visual illusions, and the interpretation of homographs [15]. Moreover, these tasks were shown to be very sensitive to the methodology used, such as the task directions.

It is currently postulated that individuals with ASD likely have stronger attention to detail than the neurotypical population, but that they are still able to engage in higher-level processing with considerable support (a somewhat watered-down version of the original theory) [2]. Weak Central Coherence has been challenged over multiple studies but still persists in this weaker format in which individuals with ASD are thought to have a preference for local levels of processing, yet an ability to engage in global processing when necessary [2]. One strength of this theory is that it has been

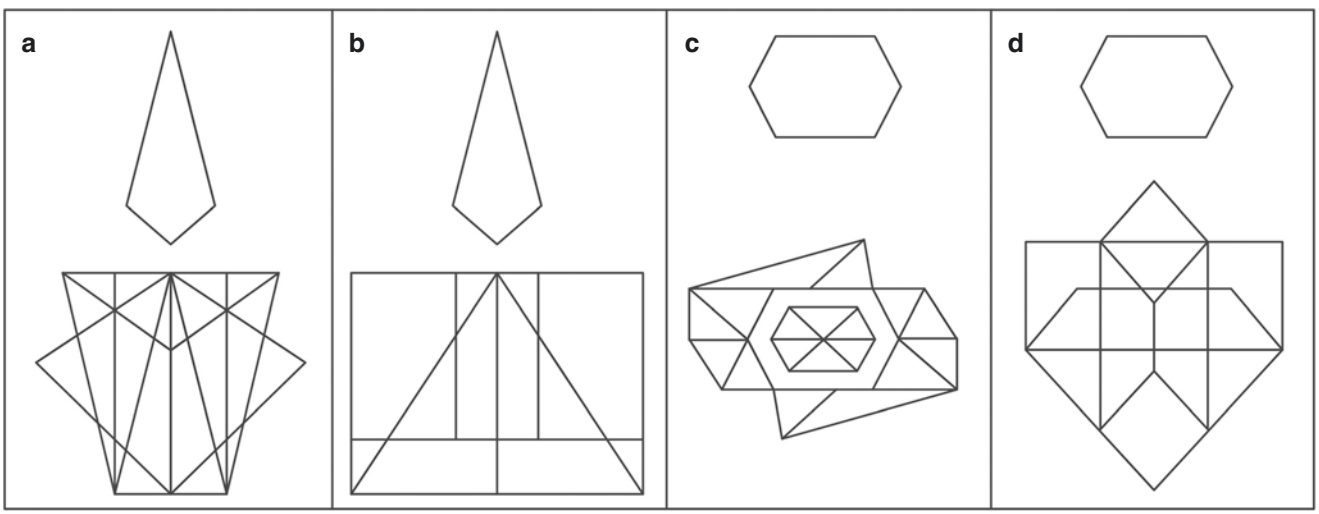

Fig. 15.1 The embedded figures task. This task involves picking shapes out of a larger picture, a task that many individuals with ASD excel at due to their attention to detail. Here, we see the task with both the target absent and the target present in the larger picture. (**a**, **c**) target present (**b**, **d**) target absent

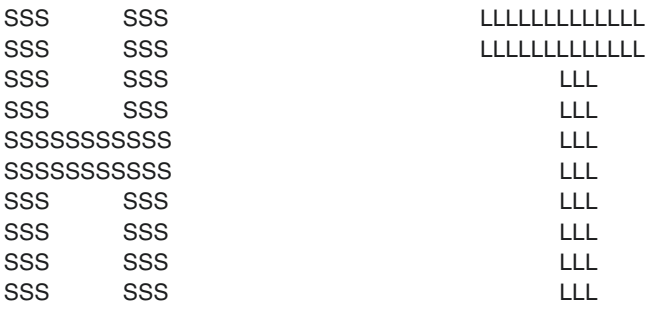

Fig. 15.2 The Navon task. While neurotypical individuals see the larger letter as a default (an H or a T), individuals with ASD often see the smaller letters that the larger letters are made up of, unless specifically prompted to identify the larger letters. This pattern of performance by individuals with ASD reflects a local, rather than global, processing bias

embraced by the autism community [2]. However, it does not comprehensively explain the social difficulties that individuals with ASD experience.

The Executive Dysfunction Theory [7] asserts that deficits in executive function are the primary cause of the symptoms of ASD. Executive functioning involves abilities such as working memory, inhibition, and cognitive flexibility that allow an individual to focus on obtaining a goal in the face of distraction. Executive dysfunction is thought to be the primary driver behind the deficits in theory of mind and has a substantial effect on routines and repetitive behaviors. This is because the false-belief task requires cognitive inhibition, that is, the child must suppress the information that s/he has to answer based on the character's knowledge. Restricted and repetitive behaviors and interests are thought to arise from a lack of cognitive flexibility.

For a long time, executive dysfunction in ASD was thought to involve primarily problems with flexibility and working memory, while inhibition was relatively unaffected. As discussed later in this chapter, it is more complicated than this. Once again executive dysfunction was not found to be correlated with autism severity in one study [16] but has been in more recent studies [13]. The lack of consistent findings across this field indicates that this theory is not reflective of the 'core' deficit in autism.

The Social Motivation Theory is the most recently hypothesized cause for the characteristic symptoms of ASD (at least the social communication symptoms) [8]. This hypothesis asserts that the majority of problems in cognition, including social cognition, stem from downstream effects of low social motivation in early childhood. That is, infants and toddlers with ASD do not attend to those around them, thus, missing out on critical learning opportunities about the social world. Thus, theory of mind is not a difficulty for individuals with ASD because they have a compromised theory of mind module, but because they have not learned from others how to effectively engage in social cognitive tasks. There has not been a lot of research conducted to test this hypothesis, but one study in younger siblings of children with ASD would seem to call it into question. Baby siblings of children with ASD are studied widely as they have a greater chance of going on to develop the disorder than children with siblings without ASD. One of these studies found that attentional problems in baby siblings were seen long before any social problems [17]. More research is needed to determine whether this theory is viable as an explanation for the phenomenology of ASD.

Box 15.1

The Theory of Mind theory of autism postulates that individuals with ASD have a core deficit in the ability to understand that others have different beliefs than they themselves have, and that others act on those differing beliefs.

Another theory proposed to explain the deficits of ASD is Weak Central Coherence Theory, which suggests that individuals with ASD are unable to convene lower-level details to obtain the big picture.

In the Executive Dysfunction theory of autism, the core deficits are postulated to be related to problems in working memory, inhibition, and cognitive flexibility.

The most recent theory is the Social Motivation theory which speculates that the difficulties that individuals with ASD experience stem from a disinterest in other people from an early age.

Which of the following statements is ***NOT*** true about Weak Central Coherence theory?

It remains valid in a watered-down form.
It is embraced by individuals with ASD.
It states that people with autism can't see the forest for the trees.
It is the most valid theory of the four theories given.

Attention

Although ASD is diagnosed in the presence of social communication deficits and repetitive or stereotyped behaviors [1], there is an expanding body of literature suggesting associated atypical attentional processes in diagnosed individuals. There are high rates of co-morbid attention-deficit/hyperactivity disorder (ADHD) within those diagnosed with ASD [18], constituting symptoms of inattention, hyperactivity, and impulsivity [1]. As mentioned earlier, a prospective approach to investigate the development of symptomatology, such as atypical visual attention, has resulted from studies of

infant siblings of children diagnosed with ASD [19]. These studies have revealed behavioral differences in the ASD phenotype early in development [19]. However, there is often heterogeneity in experimental test results within populations with ASD, likely due to the diversity of attentional tasks implemented [18], generalizability of tasks to real-world situations [20] and heterogeneity within the disorder itself. Nonetheless, many scholars argue that atypical attentional processes underlie the abnormal development of certain aspects of cognition and behavior, such as weak central coherence [21]. This section will discuss atypical attentional guidance and cognitive systems in ASD, as well as associated deficits in social attention.

Attentional Guidance Considerable evidence suggests atypical attentional allocation guided by top-down and bottom-up processes in individuals with ASD. Top-down attentional control is based on desires or goals of the individual and/or knowledge of the world [22]. For example, this type of attentional guidance would occur when searching for a friend in a crowd by looking for the yellow jacket that they are known to be wearing. Conversely, bottom-up attentional modulation is influenced by salient stimuli from the environment [22]. This type of attentional guidance would occur, for example, when a loud noise in the crowd captures attention and distracts from searching for the friend. Appropriate attentional guidance typically requires the integration of both of these processes [22]. An example of this integration is when a stimulus-driven shift of attention depends upon on an existing top-down attentional setting [23]. Unlike neurotypical individuals, salient and behaviorally relevant information often fails to capture the attention of those with ASDs (e.g., a person entering a room), while subtle and behaviorally irrelevant information tends to result in distraction (e.g., natural light entering through a window's blinds) [22].

Attentional Systems A framework developed by Posner and Peterson (1990) is frequently used for investigating attention and operates through various networks of brain areas, with each embodying a different set of cognitive processes [21, 24]. The alerting system allows maintenance of a state of vigilance that is optimal to respond to stimuli of high priority and relevance [21]. The orienting system provides the ability to attend to specific forms and locations of sensory stimuli [21]. The executive system allows top-down attentional control for observation and navigation of attentional conflict [21]. There is conflicting evidence regarding how and which of these systems are dysfunctional in ASD [21]. Although there are heterogeneous findings regarding these attentional systems, dysfunction in one or more of these networks is reliably reported in the literature [21, 25].

These atypical attentional systems have recently been postulated to underlie a visual search advantage associated with ASD. A visual search task typically involves detecting a relevant stimulus (i.e., the target) and rejecting irrelevant stimuli (i.e., distractors) after the presentation of a cueing stimulus (see Fig. 15.3). Numerous studies have demonstrated that individuals with ASD perform more quickly and/or more accurately in various visual-attentional tasks, including visual search [26]. An Enhanced Perceptual Functioning theory [27] was the dominant explanation for advantages found during certain cognitive tasks such as visual search [26] and will be discussed further during the *Perception* section of the chapter. This theory posits that over-functioning brain areas typically involved in bottom-up processing allow for enhanced low-level discrimination of stimuli (such as targets and distractors during visual search) [13]. More recent studies surrounding attentional systems have resulted in an Atypical Attention theory, comprising a tendency to overfocus (attributed to dysfunction of the alerting system) at the cost of resistance to disengagement of attention (attributed to dysfunction of the orienting system), resulting in a visual search advantage for some individuals [25]. Both of these theories relate to the Weak Central Coherence Theory with a focus on detail-oriented (local) rather than 'big picture' (global) perceptual and attentional processing, respectively [6, 14]. It should be noted that this visual search advantage is not always found experimentally and requires further investigation.

Social Attention Evidence suggests that cognitive processes, such as atypical attentional networks, may underlie the diagnostic socialization difficulties associated with ASD. Eye-tracking studies of individuals with ASD have found atypical attentional allocation, specifically in reduced attention to social stimuli (i.e., the eyes, mouths, and faces of other people) [28]. Additionally, these individuals exhibit increased attention to non-social stimuli and unusual aspects of social stimuli (i.e., the bodies of other people) [28].

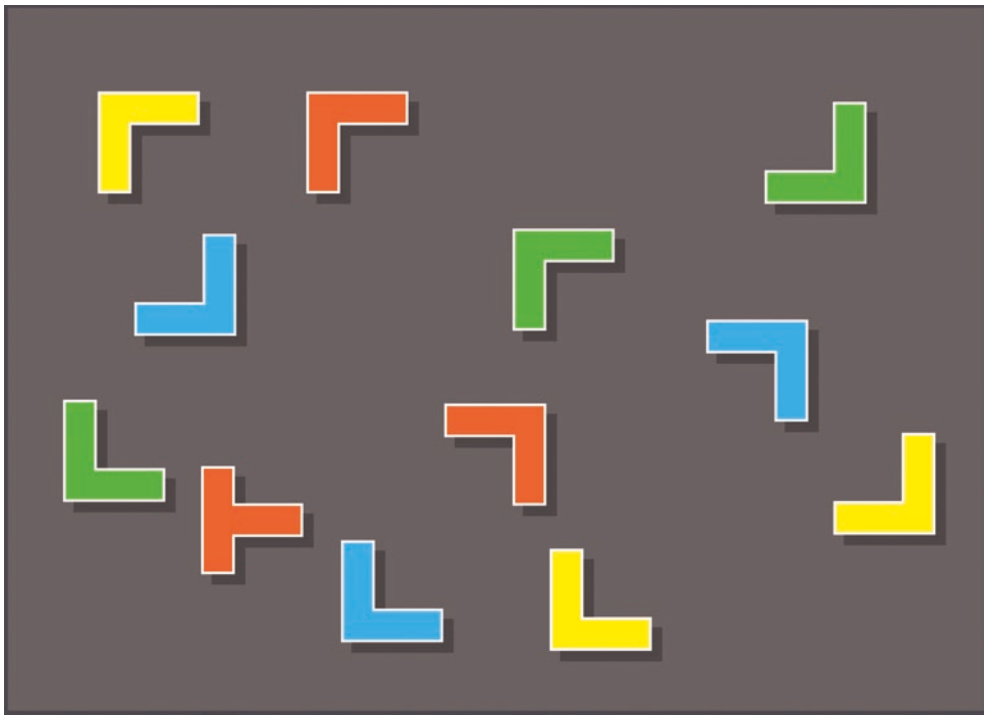

Fig. 15.3 The visual search task. Individuals with ASD often perform more quickly on this task (which is to find the T among the L's) than neurotypical individuals

It is widely accepted that individuals with ASD reliably exhibit deficits in joint attention, which refers to the capacity of an individual to coordinate attention with social partners to a specific point of focus and will be discussed further in the *Social Cognition* section of the chapter. A precursor to joint attention is gaze following, which involves attentional guidance by the direction of an observed person's gaze [29]. Attentional cueing paradigms (derived from Posner, 1980) are frequently used to study this concept but often do not capture the expected impairments in sample populations with ASD [29, 30]. A central cueing stimulus of a face is presented on a screen before the presentation of an adjacent target stimuli (such as a shape) [29]. The face acts as a congruent or incongruent gaze cue depending on whether its eyes are pointed toward or away from the target location, respectively (see Fig. 15.4) [29]. Since individuals with ASD exhibit debilitated gaze following and subsequent to joint attention, they should not display faster target detection after the presentation of a congruent social cue when compared to an incongruent social cue (as found in those who are neurotypical) [29]. Unexpectedly, performance is often comparable between neurotypical populations and those with ASD on the gaze-following task. It should be noted that as experimental paradigms become more replicative of real-world social situations, the expected effects may be more likely to emerge [20].

Box 15.2

Attention systems are postulated to be dysfunctional in individuals with ASD based on visual and social tasks, although this dysfunction is seen more clearly in ecologically valid tasks.

Which of the following is the best example of a bottom-up process?

Joint attention.
Gaze following.
Imitation.
Looking for your friend in a crowd, keeping in mind he has a red hat on.

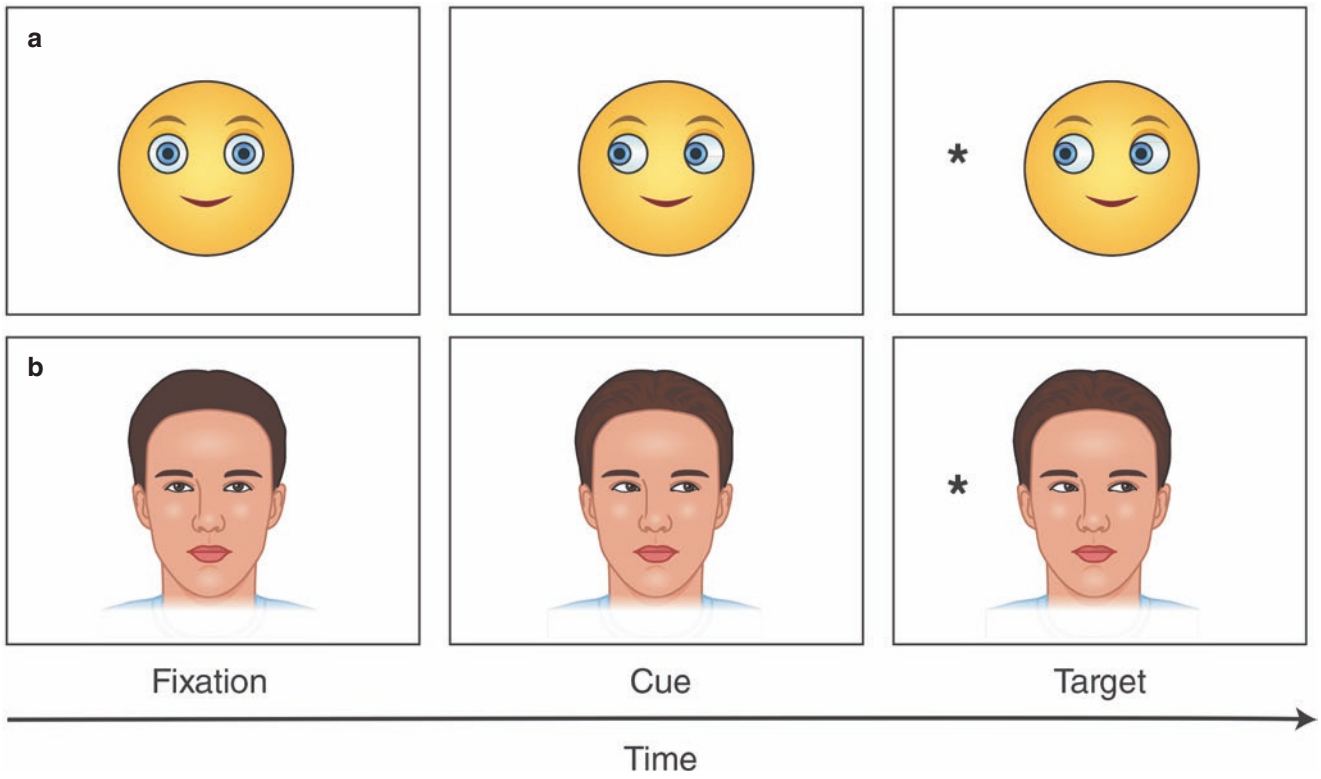

Fig. 15.4 The gaze-following task. Panel (**a**) represents a valid trial while Panel (**b**) represents an invalid trial. Performance on this task shows no differences between individuals with ASD and neurotypical individuals

Perception

A common symptom across the autism spectrum seems to be atypical behavioral responses to sensory stimuli [31]. A large number of diagnosed individuals have reported hyper- and hypo-sensitivities in multiple domains of sensory perception including auditory, tactile, and visual stimuli [31]. Sensitivity to sensory stimuli can result in distress, aggression, and self-injurious behaviors, especially in individuals who are not able to communicate their discomfort due to a lack of verbal ability [31]. Sensory-based behaviors can range from mild to severe and can persevere into adulthood [31]. Common findings in different domains of sensory perception will be reported in this section, but it is important to note that there is considerable discrepancy in neurobiological results and further research is necessary. This section will also discuss self and social perception as they relate to ASD symptomatology.

Sensory Perception Sensitivity to auditory stimuli in ASD can manifest in a number of ways, such as hyper-sensitivity resulting in discomfort to loud sounds (e.g., the recess bell at school). Although findings related to auditory perception are heterogeneous, studies have found atypical neural activity as early in the processing stream as the primary auditory cortex, suggesting delayed development [31, 32]. This area is the most highly organized processing unit responsible for acquiring and parsing sound information, which is foundational for language and communication skills development [31]. Disruptions early in the auditory pathway may be detrimental to the development of these skills.

Sensitivity to visual stimuli can manifest in many ways as well, such as hyper-sensitivity inducing discomfort to bright lights, or seeking of additional visual input due to hypo-sensitivity (for example, from repetitive hand or finger movements in front of the eyes). A theory of enhanced detail perception is often used in visual processing literature to describe an advantage during certain perceptual tasks that require the prioritization of local stimuli (e.g., visual search) [27] but also impairment in more complex tasks requiring global perception [33]. Once again, findings to support enhanced detail perception are somewhat inconsistent. A recent study found a positive correlation between presence of autistic traits in children and ability to disembed a small figure from a visual scene [33]. This finding supplements the expanding body of literature that suggests atypical visual processing associated with an ASD diagnosis but also emphasizes the importance of considering the heterogeneity of the disorder [27, 31, 33].

Abnormal tactile processing is common in individuals with ASD. Given that touch is critical to the early develop-

ment of social relationships, it is possible that this dysfunction may underlie some aspects of ASD symptomology [34]. Hyper-sensitivity to tactile stimuli can be exhibited by discomfort caused by the feeling of specific types of clothing [34]. Hypo-sensitivity can be exemplified by under-responsiveness to painful stimuli [34]. Abnormalities in response to sensory stimulation are one of the most common parental concerns for children with ASD, but neurobiological studies regarding the basis of tactile processing dysfunction have resulted in largely inconsistent findings [34].

Atypical perceptual experiences for individuals with ASD are thought to be a result of impairments in appropriately processing low-level stimuli simultaneously, the so-called multisensory integration [31]. This is evident in the 'flash-beep' illusion, which requires integration of sensory inputs across domains of auditory and visual stimuli [31]. Multiple auditory tones can be paired with a single visual stimulus to induce the perception of multiple flashes or multiple flashes can be paired with a single auditory tone to produce the perception of one flash [31, 35]. Earlier studies demonstrated that individuals with ASDs are more sensitive to the first illusion than neurotypical counterparts [31], while more recent studies have found sensitivity to only the latter illusion [35]. It is possible that this inconsistency may reflect the heterogeneity of autism; nonetheless, associated atypical multisensory integration is generally supported.

Self-Perception There is extensive evidence indicating diminished self-awareness in individuals with ASD, constituting an important factor connecting social perception and socially adjusted action [18]. Individuals with ASD often exhibit inappropriate usage of personal pronouns, indicating diminished perceptions of self-involvement [36]. When recalling past events, these individuals have demonstrated an increased likelihood to report in the third-person perspective (i.e., 'he/she') and a decreased likelihood to report in the first-person perspective (i.e., 'me') when compared to those who are neurotypical [36]. This finding indicates that these individuals are less likely to recall episodic memories from their own point of view [36]. Episodic memory difficulties will be discussed in the *Memory* section of the chapter.

Additionally, it is suggested that individuals with ASD may struggle to perceive, understand, and/or express their own emotions. Findings related to the perception of personal emotions have been persistently inconsistent in the literature, potentially due to the diverse nature of experimental methods implemented and/or a lack of ecological validity in simulated emotional experiences. A recent study examined the self-report of emotional perception based on individual life events in adolescents [37]. Neurotypical individuals were able to report more full and coherent perceptions of their own emotions and which life events provoked them, compared to those with ASD [37].

Social Perception As mentioned in the introduction, deficits in theory of mind have been posited to underlie diagnostic symptoms of ASD [5, 10]. In addition to understanding personal emotions, recognizing emotions in others is essential for socialization, as it cultivates appropriate responses [38]. Extensive evidence suggests that ASD is associated with impaired recognition of others' emotions, including slower identification, increased errors and atypical autonomic responses [38, 39]. A number of foundational abilities are required to develop the capacity to perceive emotional states of others that are known to be dysfunctional in individuals with ASD. Multi-sensory integration is necessary, as emotions are interpreted through speech as well as facial and bodily gestures [39]. The ability to divide attention and focus on relevant information is also required [39]. As previously discussed, these abilities have been found to be dysfunctional in individuals with ASD. Moreover, individuals with ASD have demonstrated atypical facial and speech stimuli processing, resulting in diminished emotional understanding [39].

In a recent meta-analysis, the authors found that emotion perception and processing studies demonstrated that individuals with ASD revealed performance almost one standard deviation below the performance of well-matched controls ($g = -0.80$) [40]; these studies were primarily conducted on perception of facial emotion. Some researchers [41] have hypothesized that impaired face processing of emotions may stem from a lack of early social motivation, as they found that facial identity and expression perception were correlated in the ASD group but not the comparison group. Studies have also consistently found reduced memory for both facial identity and emotional expression [42]. This field is not without controversy, however, as some studies have found that emotion recognition is more associated with alexithymia (the inability to understand and label emotions in oneself or others) rather than ASD symptoms [43]. Further research is required to understand the nature and neuropsychological basis of emotional recognition in individuals with ASD.

Box 15.3

Many individuals with ASD have hyper-and hypo-sensitivities in the visual, auditory, and touch systems.

Multisensory integration, or the ability to integrate stimuli coming in from different senses, may be particularly affected in individuals with ASD.

Individuals with ASD have atypicalities in their sense of self, which is evident most strongly in their difficulties with episodic memory.

The perception of emotion in others, particularly recognizing the facial expression of emotion, is another challenge for individuals with ASD.

Intelligence, Academics, and General Cognition

We have placed intelligence, academics, and general cognition all in one section in this chapter because they are strongly related to one another in neurotypical individuals (virtually no research has looked at this relation in individuals with ASD). Moreover, research in all three of these fields in ASD is sorely lacking. Given the importance of intelligence level to overall level of functioning, surprisingly little research has been conducted on the nature of intelligence in individuals with ASD. Academics is another under-studied field, with little research exploring the academic profiles of individuals with ASD. Finally, while there has been somewhat more research on general cognition in these individuals, more research is needed, particularly from a strengths-based perspective. That is, individuals with ASD seem to have some unique strengths in general cognition which lead high-functioning individuals, at least, to perform more accurately than their neurotypical peers on tests of reasoning and rationality.

Intelligence It is known that individuals with ASD have a very spiky profile, meaning that they do much better on some subtests (for example, Block Design) than other subtests (for example, Comprehension) [44]. It is possible that one of the reasons that we do not know a lot about the IQ profiles of individuals with ASD is that they are less testable than their neurotypical peers; that is, they are able to finish fewer subscales of the average IQ test, particularly in the younger years [44]. Charman and his colleagues (2011) conducted a relatively large-scale study of 156 case files from a larger longitudinal study and utilized sample weighting estimates to extrapolate percentages of children in each level of intelligence from the larger study. They found that 7% of the children fell in the severe/profound range (IQ < 35) of intellectual disability, 8% fell in the moderate range (IQ = 35–49), and 39% fell in the mildly intellectually disabled range (IQ = 50–69). About 17% fell in the below average range (70–84), 25% in the average range (85–115), and only 3% scored above average (> 115) on their IQ tests [45].

Many have argued, however that traditional IQ tests underestimate the innate abilities of individuals with ASD. For example, one study found that individuals with ASD scored 30–70 percentile points higher on a test of pure nonverbal reasoning, the Raven's Matrices, than they did on a traditional Wechsler IQ test. This pattern was not seen in the neurotypical control group [46]. More research is needed in this area to further clarify the nature of intelligence in individuals with ASD, as well as replication studies with larger samples to determine the percentages at each level of intelligence.

Academic Abilities When studies of academic profiles have been conducted, the results are generally reported at a group level with no examination of potential subgroups. One study found that 73% of their sample of children with ASD had scores on traditional math and reading tests that were highly discrepant from their full-scale IQ scores [47]. They broke the sample into four subsamples using high and low reading and math, respectively, and found that reading comprehension was the most pervasive difficulty, which fits in with most previous findings. Interestingly, poor reading comprehension was related to social and communication difficulties in these children [47]. Reading comprehension has been found to be consistently impaired across studies; however, more research is needed to investigate what underlying skills predict different patterns of academic abilities to enable us to intervene early to teach children these skills.

General Cognition There are a number of difficulties that individuals with ASD experience in terms of general cognitive abilities. They have difficulties in processing speed, generally scoring lower than neurotypicals on tasks requiring speeded performance [48]. These processing time difficulties have been related to motor performance deficits; that is, they simply cannot move their hands as quickly as typically developing individuals [48]. A recent meta-analysis found that processing speed was delayed by over half a standard deviation in individuals with ASD (Hedges $g = -0.61$) [40].

This same meta-analysis found impaired reasoning and problem-solving in ASD groups (Hedges $g = -0.51$). However, the researchers did not examine how many of these tasks were timed which might have been causing the observed deficits. Numerous studies have found that individuals with ASD have problems making decisions and solving problems. However, they are actually more rational than neurotypical individuals, relying more on data and not falling back on heuristics, as neurotypical individuals are so likely to do when problem solving [49–51]. DeMartino and his colleagues found that individuals with ASD were less sensitive to a contextual frame; that is, neurotypical individuals were more likely than individuals with ASD to respond more negatively to rewards framed in terms of a loss than in terms of a gain [50]. In another study, individuals with ASD were found to take more time to make a decision regarding a task, but this was because they sampled more information and behaved more rationally [51]. The reliance on rationality and sampling may not give them an advantage when processing information quickly with little information to sample from (such as during social interactions with a relative stranger).

Box 15.4
Intelligence in autism varies from those who have a profound intellectual disability to those who are of above average intelligence, although the latter is relatively rare.

Reading comprehension is the most commonly found difficulty for children with ASD in the academic realm.

Rationality and decision making may be an area of relative strength for individuals with autism, with several studies suggesting that they are more rational than typically developing peers.

Memory

Memory is a critical ability without which we would be unable to function in the world. Anecdotal reports suggest that many individuals with autism seem to have outstanding memories, knowing everything there is to know about their restricted interests. However, the research does not always reflect these anecdotal reports. With regard to memory, most researchers agree that non-declarative memory, such as procedural memory and associative learning, is tied closely to mental age level [42]. This means that individuals have non-declarative memory skills that are commensurate with their level of intelligence. Declarative memory (i.e., verbalizable memory, or that which can be declared) appears to be affected more in individuals with ASD with a low IQ than those with higher IQ [42]. However, this does not indicate that average IQ individuals have no declarative memory difficulties.

There are two types of declarative memory: episodic and semantic. Episodic is memory for events that have been personally experienced (remembering) and semantic is memory for facts and information (knowing). Episodic memory appears to be more impaired than semantic memory in individuals with ASD [52]. Even metamemory, the ability to reflect on whether you remember something or not, seems to function better in semantic memory than in episodic [53].

Immediate free recall of a list of words, for example, seems to be intact in individuals with ASD, but delayed free recall is impaired [42]. Delayed free recall is thought to be an episodic task, as it requires one to remember the experience of learning those words. Other tasks also assess episodic memory, such as remembering whether oneself or the experimenter performed an action. Interestingly, one study [54] found that children with ASD showed a typical 'enactment effect,' that is, the ability to better remember actions that were performed by the self. However, when asked if it was the experimenter or they themselves who performed the task, poorer performance was demonstrated compared to well-matched neurotypical comparison children. As mentioned earlier, many individuals with ASD have enhanced semantic memory and can remember innumerable facts about their restricted areas of interest. Their problems with episodic memory may stem from a poor sense of self, or executive functioning difficulties which will be described next.

Box 15.5
While non-declarative (nonverbal) memory is commensurate with intellectual ability, memory for facts (semantic memory) is a relative strength for those with ASD, while remembering situations (episodic memory) is quite difficult for them.

Executive Functioning

As mentioned earlier, executive functioning was once posited as the core deficit in ASD. While few still subscribe to this notion, there is no doubt that at least, some aspects of executive functioning are impaired in most individuals with ASD. Executive functioning tasks allow us to flexibly and consistently reach our goals and is composed of many diverse but related skills such as working memory, cognitive flexibility, cognitive and behavioral inhibition, planning, and generativity.

Working memory is an aspect of executive functioning which involves being able to manipulate information while holding it in mind. In a recent meta-analysis [55], working memory was found to be moderately impaired for both visual and spatial tasks; indeed, this was even found when studies were taken out which included children with co-morbid attention-deficit/hyperactivity disorder. This is in contrast to two earlier reviews which posited that spatial working memory was impaired to a greater extent than verbal working memory [56, 57]. Interestingly, in another recent meta-analysis, the authors found that working memory did not show a significant difference from well-matched comparison participants [40]. These contradictory findings, even from one meta-analysis to another, reflect the lack of certainty in the field, likely due to the large heterogeneity of the population.

Desaunay et al. (2020) found that all aspects (except inhibition) of executive functioning were impaired in their meta-analysis; however, when they eliminated studies that included children with co-morbid ADHD, working memory, flexibility, and generativity were the only domains that remained significantly different from comparison individuals [55]. Demetriou and colleagues (2019) conducted a meta-analysis of only

high-functioning individuals with ASD (average IQ or above) and found moderate overall effect sizes across all aspects of executive functioning, including working memory [58].

Despite these moderate effect sizes across studies, individual studies find a great deal of variability in executive functioning capability [59]. There are many factors that affect performance on executive functioning tasks in individuals with ASD. Tasks with greater cognitive capacity requirements, tasks assessing multiple aspects of executive functioning at once, and tasks that lack structure, explicit instructions, and have arbitrary rules have all found to effect performance negatively in individuals with ASD [48, 56, 59].

Kenworthy and her colleagues (2008) have argued that although executive functioning is not the core deficit in ASD, it plays a large role in the difficulties that individuals with ASD experience [48]. Moreover, she argues that executive functioning is always impaired in the real world. Indeed, in Demetriou and colleagues' (2018) meta-analysis, they found that when using a parent-reported questionnaire of executive functioning in daily living (the BRIEF), Hedges *g* was a whopping −1.84 (almost two different from neurotypical comparisons) [58].

Box 15.6

Experimental measures of executive functioning have tended to find moderate impairments across the board in individuals with ASD while everyday life is severely impacted by these difficulties.

Which of the following statements are ***MOST*** true about executive functioning?

It involves many different components.

It is only used in rare situations.

In ASD, it is most impaired in real-life situations.

It is rarely impaired in individuals with ASD.

Furthermore, executive functioning has been correlated with many other difficulties that individuals with ASD experience. Theory of mind has been found to be correlated with executive function in multiple studies [e.g., [59]]. Adaptive skills (social, communication, and daily living skills in the real world) have been found to decrease in adolescence, and executive dysfunction is a strong predictor of this [60]. Restricted and repetitive behaviors, which are core diagnostic symptoms, have been found to be predicted by impaired inhibition in individuals with ASD [61]. Clearly, executive function difficulties play a key role in how many individuals with ASD act on their world.

Social Cognition

Social cognition, which includes multiple processes, is clearly impaired in individuals with ASD. While some individuals with ASD are able to pass experimental tasks relating to social cognition, their ability to navigate the social milieu is, by definition, impaired [1]. Social cognition includes abilities such as joint attention (the ability to follow or direct another's gaze to an object), imitation, and theory of mind (among others not discussed here such as pretend play, emotional and face processing, and intention understanding). We will discuss each of these in turn.

As noted earlier, gaze following appears to be intact in young children with ASD, which is perhaps not surprising as it appears very early in development in neurotypical children and is thought to be an automatic, hard-wired process [62]. However, gaze-following studies are conducted in highly controlled experimental paradigms where young children have virtually nothing else to look at. In real life, gaze following and joint attention may not occur as easily. Indeed, one study retrospectively examined home videos and found that their entire sample of toddlers who were later diagnosed with ASD was showing impairments in joint attention by 2 years of age [63]. As children with ASD become older, they may respond to joint attention, but do not initiate it [41]. This is important because children learn language through the initiation of joint attention to an object, by pointing and implicitly requesting their caregiver to label that object [64, 65].

Most studies have found that imitation is impaired in individuals with ASD. In a recent meta-analysis, Edwards (2014) found that Hedges *g* between typical and well-matched groups was −0.81 for the ASD group on elicited imitation [66]. This study found that individuals with ASD experienced far more difficulty in imitating the *form* of the actions than with their ability to imitate the *end point* of the action [66]. These findings are all the more striking given that the behavioral therapies generally used to treat children with ASD rely primarily on imitation to teach these children. Another study found that individuals with ASD do not recognize when others are imitating them [67]. Although most theorists believe that there is something inherently social about the imitation deficit in ASD, others have argued for a deficit in visual attention [68], or visuomotor integration (the ability to combine information from the eyes with information from the hands) [69]. Perhaps not surprisingly, imitation has been associated with language ability in children with ASD [70].

As mentioned in the introduction, theory of mind deficits is not universal in ASD, but a large majority of individuals with ASD exhibit them. A recent meta-analysis found an

effect size of greater than one standard deviation for individuals with ASD compared to well-matched controls (Hedge's $g = -1.15$) [71]. Some theorists have hypothesized that individuals with ASD learn to 'hack out' (using language and logic) the standard tests of theory of mind such as the false-belief task and others, but that their theory of mind does not arise spontaneously as it does in neurotypical individuals [11, 72]. Moreover, this hacking out of the answers involves different brain areas than neurotypicals use for theory of mind tasks [72]. Interestingly though, the recent meta-analysis found that theory of mind ability was not correlated with verbal IQ or full-scale IQ of the samples but was negatively correlated with performance IQ. That is, within studies (but not necessarily individuals), those samples that had a higher nonverbal IQ performed more poorly on theory of mind tasks. This thought-provoking finding needs to be further investigated. It should be noted that individuals with ASD are not uniquely impaired on theory of mind tasks; those with intellectual disability are also deficient in these tasks [73]. Social cognition is clearly impacted across individuals with ASD, and these difficulties are related to the social communicative core symptoms of ASD. Now that we have addressed the social aspects of social communicative difficulties, we will turn to the more communicative aspects.

Box 15.7
Most areas of social cognition are found to be consistently impaired in individuals with ASD; even when individuals with autism are able to reason through the tasks, they do not have the same intuitive understanding of other people that neurotypicals do.

Social cognition does ***NOT*** include which of the following abilities?

Knowing all the other person's personal preferences.
Pretend play.
Understanding the intentions of others.
Understanding the actions of others.

Language

Although it used to be that half or more of children with ASD were considered to be nonverbal [4], this is no longer the case. A recent study found that 10% of their large sample was nonverbal, 15% minimally verbal (using only single words), and 75% had phrase speech [74]. This likely stems from the increasing accessibility of early behavioral intervention, as this intervention strongly predicts later language outcomes [75]. Language ability is strongly predicted by nonverbal IQ [64]. Language ability is also negatively predicted by the degree of autism symptoms [76]. It is always important to keep in mind, however that individuals with ASD range from the completely mute to the highly fluent.

Early intervention is important, as a meta-analysis found that there was far less progress in language development for those over the age of nine [77]. Other researchers discovered that no matter what the level of language of the child, they developed in parallel after age six, and the only real differences in trajectory occurred before this age [75]. Another study that points to the importance of early intervention is one that found whether or not the toddler had words by 24 months was the best predictor of language ability in middle childhood [78].

Phonology and Syntax Phonology, or the production and comprehension of the smallest units of language, is largely intact for most individuals with ASD, although there may be a subset of individuals with impaired phonology [79]. Some have argued that syntax (grammar) is largely intact [80], but many studies have found that individuals with ASD have speech that is syntactically less complex [79]. This is likely because individuals with ASD are less likely to use language for social communication purposes and more likely to use language just to get their needs met [81]. However, it is highly likely that there is a small subgroup of individuals with ASD who have syntactic problems that are reflective of a specific language impairment [82]. In a longitudinal study of young children with ASD, Naigles and her colleagues have found that syntax develops in a roughly mental-age-appropriate manner, while semantics tends to lag behind mental age [80].

Semantics While there are some who argue that semantics (the ability to understand the meanings of words) are intact [83], extensive research has found that semantics are impaired in individuals with ASD [79, 80, 84]. Given that social interactions (such as joint attention) are key in learning the meanings of words, the early social difficulties that children with ASD experience likely interfere with their learning of meaning [80]. Individuals with ASD simply do not seem to organize words in the same way in their brains [79, 80], and there is much evidence for this.

Individuals with ASD are less affected by semantics in memory, that is, they are less prone to the memory illusion of remembering a strongly related word like *sugar* when given a list of words to remember like *cake, sweet, candy, ice cream,* and *desserts* [79, 85]. They are impaired in the realm of categorical induction, which means that children with ASD do not induce properties of a category to other members of that category in the same way that neurotypical children do [84, 86]. When asked to generate a list of all the animals they could think of, children with ASD were more likely to come up with less prototypical words like 'aard-

vark' and 'ostrich' than were neurotypical children [87]. Children with ASD were found not to be affected by being exposed to a semantically related 'priming' word when asked to decide whether a string of letters presented was a word or not. For example, when asked if the word 'beef' was a word, unlike the neurotypical children, their reaction time was not faster if they had been shown the word 'cow' shortly before 'beef' [88]. Finally, children with ASD were found to use fewer mental state verbs (e.g., 'think,' 'know,' 'believe') in their narratives, and the number of mental state verbs used correlated with theory of mind [84, 89].

Pragmatics Pragmatics is the area of language that everyone agrees is universally impaired among individuals with ASD [79, 80, 83]. Pragmatics refers to the social *use* of language and includes various aspects of language such as humor, non-literal language, inferencing, informativeness, relevancy, levels of politeness, and style of speech. Pragmatic issues are one of the most stigmatizing symptoms for individuals with ASD [90] as they simply do not speak like those who are neurotypical. They have idiosyncratic ways of expressing intentions, fewer intentions expressed, and an unusual directness to their speech [90]. Sabbagh (1999) hypothesized that the difficulties that individuals with ASD experience in pragmatics are due to their lack of ability to understand communicative intentions [91]. Indeed, pragmatic ability has been found to be strongly related to measures of social cognition [83, 92].

Numerous studies have found that individuals with ASD do not understand humor or non-literal language, and have difficulties making inferences [79]. Children with ASD tend to speak in what is known as a pedantic style, sounding like 'little professors' even at a young age [79]. They are poor at what are known as Gricean maxims; that is, avoid redundancy, and be informative, relevant, thoughtful, and polite when engaged with a conversational partner [92]. Individuals with ASD have more difficulty telling a story from a wordless picture book, including fewer story elements (such as setting, character, conflict, and resolution), and giving fewer causal elements of the story [84, 93, 94]. Capps and her colleagues (1998) found that children with ASD fail to respond to questions and when they do respond, they offer less new information and talk less about their personal experience [95]. Indeed, one naturalistic study found that they could not even answer their parents' questions at the dinner table [96]. Of course, some of these difficulties might stem from difficulties in syntax or semantics as well. It is imperative that we develop more large-scale longitudinal studies across language domains so we can see how the different types of language affect one another across development [80].

Box 15.8

Pronunciation (phonology) and grammar (syntax) are relatively unimpaired in ASD, although there is likely a subgroup of individuals with ASD who have impairments in these areas similar to those of individuals with specific language impairment.

Semantics (understanding the meaning of words) are often found to be different among individuals with ASD—they simply do not have the same organization of meaning in their brains as neurotypical individuals do.

The one area of language where individuals with ASD are universally impaired is pragmatics, or the social use of language.

What is the most universally impaired aspect of language in ASD?

Phonology
Syntax
Semantics
Pragmatics

Limitations and Future Directions

As we have been discussing throughout the chapter, one of the biggest issues in autism research is the enormous heterogeneity of the population [2]. There is a saying among autism researchers that, 'if you have seen one child with autism, you have seen one child with autism.'. As far back as 2003 [97], researchers were talking about the fact that there are likely many etiologies and multiple different genes that are causing this behaviorally defined disorder. If those were not difficult enough, autism encompasses a much broader spectrum than it did 20 or 30 years ago [2]. Individuals who in the past may have only been diagnosed with intellectual disability, or just been thought of as extremely odd, are now getting a diagnosis on the autism spectrum. Evidence for this is seen in a recent meta-analysis which found that effect sizes for all different types of comparison studies with ASD as the group of interest have been coming down over the last number of years, indicating that the ASD population is becoming closer to the comparison groups that they are paired with [98]. Aspects of cognition are important in that they may be able to give us more objective data to group our participants into—this should be done in the future. For example, researchers could study individuals with problems in seman-

tics versus those without to examine whether there are other behavioral differences of interest or even brain or genetic differences.

We must be careful, however, not to leave some individuals with ASD out of our testing, Russell and colleagues conducted a very illuminating meta-analysis in 2019 [99]. They took all of the articles for from the top three impact-factor journals dedicated to autism research for the entire year of 2016 in all areas of research. Despite the fact that they excluded any article with the term 'high-functioning' in the title, they found that 94% of all of the samples amalgamated together were high-functioning or had IQ in the average range. There are many reasons for this: individuals with ASD who are lower functioning are more difficult to test, their families are generally more reluctant to bring them into the lab, etc. However, with the exception of epidemiological studies that were more reflective of the ASD population at large, research is virtually neglecting over half of the population of individuals with ASD [45]. Research needs to be designed so that lower-functioning individuals with ASD can contribute increased information to the research field.

Research in the autism field has been notorious for very small sample sizes. Until the early 2000's, it was not uncommon to see studies of individuals with ASD and comparison groups with no more than 10 individuals in each group. As the prevalence of ASD has been increasing [100], sample sizes have gotten larger. However, the requirements of the research questions have changed to necessitate larger numbers of participants, as we move beyond simply looking at group differences and begin to explore the associations between variables and which factors predict group differences. In other words, multiple regression requires much larger sample sizes than simple *t*-tests. Some researchers appear unaware of this statistical truism and try to look at numerous predictors of an outcome with only 40 or 50 participants in their study. Many researchers in the field have moved toward collecting data through multi-site consortia which improves the ability to collect large enough samples. However, these consortia can be difficult to become a part of, particularly for junior investigators, and more effort needs to be put into making these consortia more accessible.

Individuals with ASD can be very difficult to test, given their reduced social motivation to please the experimenter [59, 79]. One way to get around this is to administer all tasks on a computer, which individuals with ASD may find more enjoyable [59]. Indeed, studies have found that individuals with ASD tend to do better on computerized tasks than those given in person [101]. More attempts need to be made to ensure that individuals with ASD are performing at their optimal level when undertaking research studies.

Box 15.9

Research in the field of cognition is hampered by the great heterogeneity of this population, likely caused by the large number of different genetic causes of ASD.

Recent research in the field of autism has focused almost entirely on high-functioning individuals—this needs to change.

Small sample sizes impede researchers from reaching solid conclusions from their research.

Given the low social motivation of individuals with ASD, it might be better to present experimental tasks on the computer whenever possible.

The field needs more longitudinal studies, where we can begin to untangle the nature of the effects that the social deficits have on cognitive functioning over time.

As mentioned earlier, we are now at the stage where we have begun to better understand the differences between individuals with ASD and neurotypical individuals and what is important is to understand *why* those differences occur. To do this, there are several things that need to change surrounding the majority of autism research studies. We need to begin investigating the social and cognitive aspects of ASD in tandem to investigate the effects they have on one another [102]. It is important to start as early as possible (the baby sibling studies are ideal for this) to take a truly developmental approach. We need more longitudinal studies, to investigate how different aspects of social and cognitive factors interact with one another over time. We need to ensure as much as possible that our experiments are ecologically valid. That is, while still maintaining experimental control, we need to test how individuals with autism perform in real life. Finally, we need to begin to investigate the effects of treatment on social and cognitive development. Virtually no studies in the autism field control for treatment effects, in part because they are so difficult to measure. We must at least try; however, because there are still large differences between children in the amount and types of treatment that they receive. While we know a great deal about autistic cognition, there is still an enormous amount to learn.

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The Neuroradiology of Autism: Framing Neuroimaging Investigations of the Autistic Brain Based on the US NIMH Research Domain Criteria

16

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Learning Objectives

- Being endowed a bird's-eye view of the neuroimaging/neuroradiological findings associated with autism spectrum disorder (ASD).
- Learning the basic nomenclature of the US NIMH Research Domain Criteria (RDoC) systems and interpreting the diverse neuroimaging findings associated with ASD based on the RDoC framework.
- Understanding the potential factors contributing to the heterogeneous findings in the neuroimaging literature of ASD.
- Recognizing major methodological caveats and prospects of neuroimaging studies in ASD and related neurodevelopmental disorders.

Highlights

- We provide organizing principles based on the RDoC framework to interpret the diverse neuroimaging/neuroradiological findings associated with ASD.
- Altered brain structure and function associated with ASD not only involve the Social Processes Systems but also all other Systems within the RDoC framework.
- Demographic, developmental stages and clinical features contribute to the heterogeneity of neuroimaging findings of ASD.
- Neuroimaging methodological caveats partly contribute to inconclusive findings.
- Future studies would benefit from larger sample sizes with more inclusive recruitment, better neuroimaging denoising strategies, and novel conceptual frameworks, brain metrics, as well as analytic methodologies.

Introduction

Significant gains in insights into neurodevelopmental alterations in brain structures, functioning, and connectivity have been seen in the past two decades. A major contributor to this increased understanding is the development of neuroimaging techniques, especially magnetic resonance imaging (MRI, including structural MRI, sMRI, functional MRI, fMRI, and diffusion-weighted imaging), proton magnetic resonance spectroscopy (^{1}H-MRS), alongside molecular imaging (including single-photon emission computed tomography, SPECT, and positron emission tomography, PET). sMRI provides contrast between gray (GM) and white matter (WM) tissues, which can be used to estimate anatomy such as volume, cortical thickness, surface areas, and gyrification [1]. fMRI exploits differences in the ferromagnetic proper-

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ties of oxygenated and deoxygenated blood to generate an indirect measure (the blood–oxygen level dependent, BOLD, signal) of neural activity [2]. fMRI measures localized brain regional activities and the connectivity of distributed brain networks (e.g., functional connectivity based on statistical interdependence of BOLD signals between the investigated regions). Diffusion-weighted imaging estimates differences in the magnitude of diffusion of water molecules within the brain, which is sensitive to changes in WM microstructure, and could estimate structural connectivity [3]. For biochemical molecular imaging, ^{1}H-MRS uses the nuclear magnetic resonance properties of hydrogen atoms to quantify low-molecular-weight neurotransmitters and metabolites within the selected brain areas [4]. PET/SPECT estimate neurotransmitter release and occupancy, glucose metabolism alongside oxygen consumption by quantifying density of a specific targeted protein to which a radiotracer binds [5]. These neuroimaging approaches allow for in vivo investigation of the autistic brain.

The goal of this chapter is not to provide an exhaustive review of the rapidly evolving neuroimaging findings. Rather, we provide organizing principles for framing the diverse neuroimaging/neuroradiological findings to date. As the heterogeneity in autism spectrum disorder (ASD) may be associated with multi-level processes cutting across frequently co-occurring diagnoses [6], we use the US NIMH Research Domain Criteria (RDoC) framework [7] to summarize the neuroimaging findings. Specifically, we map neurobiological alterations associated with ASD onto six RDoC domains, including Negative Valence, Positive Valence, Cognitive, Social Processes, Arousal/Regulatory, and the recently added[1] Sensorimotor systems. This approach highlights the transdiagnostic lens across neurodevelopmental and psychiatric conditions [8–12], which is necessary in clarifying the neurobiological nature of ASD and other behavior-based diagnoses. To preempt nomenclature ambiguities and to conform brain structural segregation with their functional ontologies, the Yeo-Krienen seven networks model [13] is adopted as a reference framework alongside localized neuroanatomical labeling.

Neuroimaging Findings Based on RDoC Domains

Negative Valence Systems

Constructs within the Negative Valence systems are negative emotionality and responses to aversive environments, such as fear, anxiety, and loss-related behaviors. The key neural correlates are within the fronto-limbic circuitry including the amygdala, anterior insula, and lateral orbitofrontal cortex (OFC) [14], which involve bottom-up limbic reactivity to affectively salient stimuli, top-down prefrontal activities, and their interactions. Beyond core autism characteristics, individuals with ASD frequently present with co-occurring anxiety and depression symptoms or diagnoses [15] alongside emotion regulation difficulties, especially heightened negative affect [16], and have difficulty recognizing emotional status of sad and fearful faces [17]. fMRI studies of Negative Valence in ASD show hyperactivation in the thalamus, caudate, and hypoactivation in the hypothalamus [17] and fusiform facial area [18] during emotional-face processing. However, results of amygdala responses to negatively valenced expressions in ASD are mixed [17, 18]. Interindividual variations of anxiety in ASD appear to dimensionally modulate amygdala activity to faces with negative affect [19, 20]. During negative cognitive reappraisal, autistic children demonstrate decreased capacity to down-regulate amygdala activity [21, 22]. During peer exclusion, hypoactivity in the anterior insula and ventral anterior cingulate cortex (ACC) are consistently identified in ASD [23]. Structurally, OFC and ventromedial prefrontal (vMPFC) volume are related to self-regulation capacity, especially monitoring and modifying negative effect, across autistic and neurotypical youth [24]. Amygdala volume is negatively correlated with anxiety symptoms in ASD [25].

In general, a relatively small number of neuroimaging studies have focused on Negative Valence systems, yielding an emerging picture of compromised affective circuitry in ASD (see [26] for alternative accounts), which may be associated with the high co-occurrence of negative valence symptoms and categorical diagnosis (e.g., anxiety disorders [27]) in the autistic population.

Positive Valence Systems

Positive Valence systems encompass neural circuits related to motivation, reward processing, and reinforcement habit formation behaviors. Involving salience detection and valence processing, positive valence is specifically associated with activity in the vMPFC/rostral anterior cingulate cortex (ACC). It also implicates more valence-general processing regions nonetheless, such as nucleus accumbens, amygdala, thalamus, anterior insula, dorsomedial prefrontal/dorsal ACC, ventrolateral prefrontal cortex, and supplementary motor area [14]. Atypical social motivation [28] provides a complimentary framework to classic conceptualizations of social communication and social cognitive atypicalities in ASD (also see 'Social Processes Systems' section below). It hypothesizes that in autistic children, low social orientation and motivation in the first

[1] https://www.nimh.nih.gov/news/science-news/2019/sensorimotor-domain-added-to-the-rdoc-framework.shtml

few years of life may have cascade effects on impoverished social experiences, resulting in an atypical development of social cognition and social skills. Alterations of the balance between social and nonsocial motivation systems may in part account for restricted interests and repetitive behaviors (RRBIs) as well [29]. On the other hand, such alteration could be an alternative and adaptive developmental process with an atypical starting state of attention allocation (i.e., focal attention, repetition, and withdrawal from social stimuli) underpinned by neurobiological differences (e.g., synaptic processing) in autistic vs. typically developing children [30].

A recent fMRI meta-analysis [31] reveals consistent findings of atypicality in mesocorticolimbic circuitry in response to social rewards in ASD, i.e., hypoactivity in the bilateral caudate and ACC, and hyperactivity in the right insula and putamen. Hypoactive caudate, nucleus accumbens, and ACC in responses to nonsocial rewards, including monetary rewards and restricted interests, have also been found in individuals with ASD [31]. By disentangling 'wanting' phase (anticipatory drive) versus 'liking' phase (pleasure effect of reward), the ASD group shows striatal hypoactivation during wanting but hyperactivation during liking of social stimuli [31]. Using resting-state fMRI, autistic children show increased functional connectivity of striatal regions, specifically caudate and putamen, which are involved in positive affect processing, with heteromodal and limbic cortices, specifically anterior insula, superior temporal sulcus [32], and ACC [33]. Functional connectivity between regions processing Positive Valence is related to social-communication deficits and RRBIs in ASD [33]. Underconnectivity between voice-selective cortex and reward circuitry is reported in autistic children [34], potentially reflecting why they have a hard time gaining pleasurable experiences in response to social stimuli. Further, both cross-sectional [35] and follow-up [36] sMRI studies show that the rate of basal ganglia growth in autistic youth is correlated with RRBI intensity. A mega-analysis in a multinational sample shows reduced nucleus accumbens volume in individuals with ASD [37]. Reduced expression of GABA (a main inhibitory neurotransmitter) receptors, as identified by PET, is also noted at bilateral nucleus accumbens and amygdala in autistic adults [38]. Altered glutamate metabolism, as quantified by ^{1}H-MRS, is found in ACC and striatum in individuals with ASD, despite contradictory directions across studies [4].

In sum, alterations to Positive Valence systems, as represented by atypical reward processing, may in part contribute to both the development of RRBIs and social-communication difficulties.

Cognitive Systems

Cognitive systems encompass the diverse array of cognitive processes, including attention, perception, declarative memory, language, cognitive control, and working memory. Atypical processing or impairments are often reported in autistic individuals [15, 39, 40].

Attention, Cognitive Control, and Working Memory

fMRI studies of executive function, comprising attention, cognitive control, and working memory have consistently revealed anomalous activation in frontal, parietal, and striatal circuitry, including ventro- and dorsolateral PFC, dorsal ACC, superior and inferior parietal lobule, and the basal ganglia, in individuals with ASD [18]. These regions largely correspond to the frontoparietal, dorsal attention, and ventral attention (also known as Salience) networks [13]. Administration of a single dose of citalopram, a selective serotonin reuptake inhibitor, modulates (largely abolish) this atypical brain activation during sustained attention and inhibitory control in autistic adults [41]. Notably, the majority of studies indicate frontostriatal hyperactivations, alongside recruitment of other brain regions typically not involved in cognitive control. Together with the finding that striatal resting-state functional connectivity in ASD exhibits ectopic hyperconnectivity with heteromodal and limbic regions [32], this evidence might reflect neurofunctional compensation for cortical insufficiency in the Cognitive systems. A large-sample resting-state fMRI study [42] reports ASD-associated hyperconnectivity at the large swaths of the frontal and parietal cortices, which encompass a majority of the frontoparietal, dorsal, and ventral attention networks. Brain states discriminability between task-evoked and resting states among functional network configuration of these cognitive control networks, alongside default-mode network, is related to RRBIs in ASD [43]. Baseline resting-state functional connectivity [44] and structural connectivity [45] among these networks are predictive of trajectories of autistic phenotypes. Short-distance structural connectivity in the frontal [46–48], parietal, and temporal regions [47] is reduced in ASD and related to core symptoms. Finally, the microstructural property of the corpus callosum, connecting these circuitries across both hemispheres, and superior longitudinal fasciculus, connecting ipsilateral frontoparietal regions, are the most consistently reported atypical WM tracts in autistic individuals [3, 8, 9, 49–51]. This atypical structural connectivity is associated with cognitive performance [51].

Perception

Consistent with neuropsychological evidence, fMRI studies in ASD individuals show atypical responses in primary sensory regions across modalities, alongside typical responses in multisensory integration cortices (e.g., intraparietal sulcus, at which local sensory signals are integrated into a global percept) [40]. This suggests that autistic sensory traits, such as a bias towards local over global features, may originate from enhanced lower-level processing. Increased trial-by-trial variability of evoked responses at the visual, auditory, and somatosensory cortices is also found in autistic individuals [40, 52]. A ^{1}H-MRS study shows reduced GABA in the auditory and somatosensory cortices in autistic individuals. A link between GABA levels in visual cortex and the strength of visual inhibition, which contributes to binocular rivalry, is reduced in ASD [40]. Further, several resting-state fMRI studies show altered connectivity within the sensory network and its connection to other brain systems. For example, a large-scale study found ASD-related alterations in between-network connectivity mainly affect visual, sensorimotor, and cerebellum networks [53]. Another study found consistent ASD-related hypoconnectivity at the sensorimotor and temporal (auditory) cortices [42]. In the Autism Brain Imaging Data Exchange initiative (ABIDE) [54, 55] data set, increased interactions between the sensory and thalamus and basal ganglion systems are related to increased autistic symptoms [56]. Further, disruptions in connectivity transitions from sensory towards transmodal areas are identified in a large cohort of autistic individuals [57]. Sensory over-responsivity, a common autistic sensory feature, is subserved by sensorilimbic hyperresponsivity to aversive sensory stimuli due to failure to habituate [58], and also relates to microstructural property of temporal and sensory segments of the corpus callosum and superior longitudinal fasciculus [59]. Given the gate role of thalamus in sensory information processing, altered functional connectivity during exposure to sensory stimuli [60], resting-state functional connectivity [61–63], and structural connectivity [61, 62] of thalamocortical circuitries have been observed in ASD. These observations may reflect atypicalities in multisensory and local-global information integrations in ASD. Besides these cross-sectional observations in autistic children and adults, the Infant Brain Imaging Study (IBIS) found that infants who are later diagnosed with ASD have atypicalities in connectivity related to visual, somatosensory, and motor processing even before 6 months of age [64]. Lower-level sensory network characteristics may have cascade effects on the development of brain organization involving higher-level cognitive processes, and eventually the behavioral presentation of ASD [30].

Language

Typical language processes are underpinned by dorsal and ventral streams, mainly left lateralized. The dorsal pathway connects the primary auditory cortex with the interior parietal areas, linking to the inferior frontal gyrus (IFG), which subserves 'how' and 'where' processes of the auditory–speech pathway. The ventral pathway connects auditory and IFG through middle temporal and temporal pole regions, and is responsible for 'what' processes [65].

A small number of studies compared ASD individuals with and without early language delay, and a majority of them used sMRI with small sample sizes [66]. Two meta-analyses (using different analytic strategies) arrive at inconsistent findings. Yu and colleagues contrasted summary maps of DSM-IV autistic disorder-related GM alterations (autistic disorder vs. control) with those driven by DSM-IV Asperger's disorder vs. control. They found that brain areas identified by the two study sets are largely different in terms of affected regions and the directionality of differences, except shared increase in left ventral temporal lobe volume [67]. Conversely, Via and colleagues limited their examination within several regions of interest and concluded that Asperger's syndrome/disorder and autistic disorder share similar neuroanatomical substrates [68]. Another sMRI study using a male adult cohort found that history of language delay is associated with adulthood neuroanatomy in insula, temporal pole, superior temporal sulcus, and ventral basal ganglia, none of which are directly involved in the 'canonical language circuitries'. Nonetheless, current language–neuroanatomy correlation patterns are similar across ASD subgroups with or without a history of language delay [66].

fMRI investigations in ASD indicate underconnectivity and undersynchrony within language processing circuitries, such as left IFG (Broca's area), prefrontal, and temporoparietal (Wernicke's area) cortices, during semantic-pragmatic processing. Recruitment of uncanonical language-related regions (e.g., early visual parieto-occipital pathways), unexpected brain regions in response to a specific semantic component (such as hyperactivated Wernicke's area instead of typical activation of Broca's area when performing a syntactic-semantic task), and reduced or reversed leftward activation asymmetry are also noted in intellectually able autistic individuals [18, 69–71]. As quantified by regional cerebral blood flow measured by PET, reversed hemispheric dominance and reduced activation at the auditory cortex during verbal language auditory processing are also found in autistic adults [72]. Resting-state fMRI and diffusion-weighted imaging studies indicate that reduced functional and structural connectivity within the language processing

cerebro-cerebral [73, 74] and cerebro-cerebellar [75] circuitries in autistic children are related to their language impairments. Moreover, autistic individuals who have poorer structural language abilities also have greater cortical thickness and structural covariance within fronto-temporal language-related regions [76]. Baseline structural properties of language regions of autistic toddlers are related to improvement in language abilities following Pivotal Response Treatment [77]. Reading intervention is found to enhance activity in language areas and right-hemisphere language area homologs, as well as increase functional connectivity within the left-hemisphere language network during sentence comprehension, in intellectually able school-aged children with ASD [78].

Minimally verbal individuals constitute around 30% of the ASD population. Nonetheless, sparse MRI studies have focused on this distinct sub-population [65]. An fMRI study shows that minimally verbal autistic children have hypoactivated IFG and reduced connectivity between left IFG and auditory cortex during song relative to speech [79]. Extending this evidence, a longitudinal sleep-fMRI study [80] measured early cortical responses to speech and found that language-sensitive regional activity during infancy/toddlerhood could prospectively predict language developmental outcomes in children with ASD. Specifically, autistic infants/toddlers with later poor language outcome have hypoactivated superior temporal cortices in response to speech but have preserved general auditory-processing brain activation. Moreover, there is an association between the leukocyte transcriptome and neurofunctional responses to speech that higher enrichment in broadly expressed genes and ASD, prenatal, human-specific, and language-relevant genes is linked to poorer language outcome [81]. A recent resting-state fMRI study [82] demonstrates that autistic children who have low verbal and cognitive performance exhibit decreased connectivity within most major brain functional networks and reduced interhemispheric connectivity relative to autistic children without such concurrent difficulties and neurotypical children, implying aberrant brain functional segregation and integration. Finally, conforming to the double-streams framework, diffusion-weighted imaging studies indicate structural disruptions to the dorsal language pathway in minimally verbal individuals with ASD [65].

Summary of Cognitive Systems

Altogether, MRI studies across modalities show that anomalous psychophysiological performances of Cognitive systems are largely subserved by functional and structural alterations to the canonical circuitries of the constructs investigated. Recent two large-scale multi-site examinations [37, 83] also indicate altered cortical thickness at the frontal and temporal cortices, which are mainly involved in cognitive control and language constructs, in autistic individuals. Recruitment of uncanonical or unexpected brain regions and circuitries, alongside different directions in ASD-associated functional and structural alterations, might reflect neurofunctional compensation or atypical brain segregation and integration in development.

Social Processes Systems

Systems for Social Processes are responsible for interpersonal interactions through the constructs of perception and interpretation of others and self, social communication, as well as establishment of a social bond encompassing affiliation and attachment.

Atypical theory of mind, or difficulties with mentalizing, i.e., understanding of mental states in both self and others, are believed to be core to explaining the social-communication difficulties of ASD [15]. Developmental precursors of mentalizing, such as triadic social interaction (e.g., joint attention and pretend play) and dyadic social interaction (e.g., eye contact, emotion perception, action–perception mirroring, social orienting, biological motion processing, and face processing), also contribute to autistic symptomatology [15].

Most task fMRI investigations addressing social orientation, processing of social cues, and mentalizing in ASD have focused on the so-called 'social brain' regions. These regions are related to specific domains of Social Processes systems, such as the dorsal and ventral mPFC (implicated in monitoring others' and one's own mental states, respectively), the temporoparietal junction (encoding others' information such as mental states and beliefs), the posterior cingulate cortex (subserving self-referential processes), the posterior superior temporal sulcus (activated by biological motion), the inferior frontal gyrus (involved in emotional judgments), the intraparietal sulcus (guiding spatial attention in social contexts), the amygdala (recognizing emotions and other emotion-related processing), the fusiform facial area (face processing), and the anterior insula (involved in understanding internal states and mimicking social expressions) [84]. Overall, task fMRI evidence consistently indicates atypicality (mostly hypoactivity) in ASD in most of the 'social brain' regions across tasks involving social perception, social cognition, and inferring self-other processing [18, 69, 85]. Using ecologically valid task of spontaneous conversations, autistic males have abnormally increased conversation-driven interregional temporal correlations among social brain regions and their striato-cortical and thalamocortical relationships [86]. Regional cerebral blood flow at the social brain regions is also lower during socio-cognitive tasks in autistic adults [87, 88]. Acute and repeated oxytocin administrations mitigate mPFC fMRI hypoactivation during a social judgment task in autistic adults [89, 90]. This 6-week oxytocin treatment also reduces mPFC *N*-acetylaspartate and glutamate-glutamine

levels, suggesting a key role of the glutamatergic system in ASD [4, 91]. A newly developed online social cognition training is found to induce functional and structural neuroplasticity in mPFC in autistic adults; this neuroplasticity after intervention scales with performance gains on the social cognition tasks [92]. Finally, atypical mirror system (including the anterior intraparietal sulcus, premotor cortex and posterior superior temporal sulcus) is not consistently found to characterize ASD during imitation or perceiving others' actions [93].

Using resting-state fMRI, reduced network integration has been identified within and between widely distributed networks supporting core social functions, such as mentalizing, imitation (mirror system), face perception, and social-emotional processing [94, 95]. Studies investigating non-linear complexity features of the BOLD fluctuation also found reduced signal complexity in the social brain regions of autistic brain [96]. Furthermore, there is convergent evidence of reduced segregation (or differentiation) of social networks in ASD, reflected in ectopic hyperconnectivity with nonsocial regions [94]. Diffusion-weighted imaging evidence suggests that long-range fiber tracts showing ASD-associated reduced white matter integrity, include the superior longitudinal fasciculus (underpinning social communication and imitation deficits), the cingulum and inferior longitudinal fasciculus (interconnecting the regions involved in mentalizing and emotion recognition deficits, respectively) [97]. Kindergarteners with autism show improved microstructural integrity in most of these WM tracts following a year of early intervention [98].

As understanding of self in the context of others (which is critically associated with the default-mode network) is integral to reciprocal social interactions, it is not surprising that the default-mode network overlaps with the social brain regions, especially those involved in mentalizing. The extant sMRI, fMRI, and diffusion-weighted imaging literature [99] provides converging evidence for atypical default-mode network organization and development in functional and structural connectivity and morphometric property in ASD. Moreover, reduced resting-state functional connectivity of nodes within the default-mode network with other functional networks is also observed in ASD, potentially reflecting that difficulties communicating between the default-mode network and other networks may result in altered integration of socially salient stimuli into self-referential and mentalizing processes [100]. Besides the cortico-cortical connections, functional and structural disruptions to the cerebro-cerebellar circuits associated with the default-mode network, especially cortico-posterior cerebellar (Crus I/II) connectivity, are also observed in ASD [75, 101].

In sum, structural and functional disruptions to the 'social brain' network (largely corresponding to the default-mode network), as well as atypical connectivity within the system and with other brain functional systems, underlie social difficulties in ASD. Nonetheless, mentalizing is closely entwined with the Negative and Positive Valence and Control systems. We need to understand how altered dynamic interactions between multiple large-scale brain systems contribute to mental representations of socially relevant information in ASD.

Arousal and Regulatory Systems

Arousal and Regulatory systems are related to homeostatic regulation in a context-specific manner. Although a substantial proportion of autistic individuals experience problems in arousal, sleep, and circadian rhythms, only few fMRI studies have focused on the Arousal construct within the Arousal and Regulatory systems. These studies highlight altered affective arousal responses during social-emotional processing. Specifically, Tseng and colleagues [26] reported that individuals with ASD show atypical links of arousal levels with activities of the regions involved in impulse control and default-mode network, whereas arousal levels are expectedly associated with attention network activities in neurotypical individuals. Using fMRI involving pain empathy activities, Fan et al. [102] show that autistic adults use avoidant strategies to restrain affective hyperarousal, indexed by reduced anterior insular hemodynamic activation in response to passively viewing others' pain, and heightened N2 (an electroencephalographic event-related potentials index associated with affective arousal and attention novelty) response to actively making pain judgment. Using a similar paradigm, Gu and colleagues [103] identified heightened interoceptive precision, indexed by increased responses at both autonomic (skin conductance responses) and cortical (anterior insular activities) levels in intellectually able adults with ASD, potentially reflecting a recent hypothesis that failures in Bayesian inference may contribute to socio-emotional deficits in ASD [104]. The paucity of neuroimaging literature highlights the potential of studying Arousal and Regulatory systems relevant to autistic characteristics.

Sensorimotor Systems

Sensorimotor system is a newly added RDoC domain, which primarily subserves the control and execution of motor behaviors. Brain systems typically supporting motor functions consists of cortico-cortical, cortico-subcortical, and cortico-cerebellar circuitries. Frontal regions including primary motor cortex, premotor cortex, and supplementary motor area; their anatomically and functionally connected parietal regions are involved in motor planning and execu-

tion. Fronto-basal ganglia loops mediate voluntary motor initiation and inhibition. Cortico-anterior cerebellar loops subserve tuning of intended actions [105].

Autistic individuals frequently present with fine and gross motor coordination problems, anomalous motor skills learning, locomotor disturbances, dyspraxia, and rely on different strategies for motor execution [105, 106]. Despite being widely investigated behaviorally, there have been limited fMRI studies focusing on motor controls in ASD. These studies find that autistic individuals have reduced activities in the canonical motor circuits, but additionally recruit the frontostriatal circuits subserving higher-order Control systems, during oculomotor and motor tasks [69, 75, 105]. Evidence from structural and functional network analyses has identified altered cerebral lateralization in ASD with relatively unscathed or enhanced right-hemisphere functions but with reduced left-hemisphere connectivity and activity, suggesting autism-associated developmental dysmaturation in lateralization affecting left-hemisphere motor dominance [107]. Resting-state fMRI literature has observed reduced intrinsic synchrony between visual and motor systems in autistic children [53, 108], potentially contributing to social [108], action observation and praxis [105] atypicality.

Autism-associated aberrant motor circuits and compensatory use of frontostriatal systems to support sensorimotor functions may have cascading effects on later developing higher-order cognitive processes that are subserved by these 'borrowed' systems. Together with the fact that motor anomalies usually occur before phenotypic expressions of core autistic symptoms, impaired coordination of motor actions may in part impact social functioning and RRBIs in ASD [106].

Caveats in Current Understanding and Future Perspectives

Developmental and Environmental Influences

The summary of neuroimaging findings using the RDoC framework supports the notion that complex and heterogeneous phenotypes of ASD emerge from atypical brain organizations across multiple RDoC domains and constructs. Nonetheless, other two critical dimensions within the RDoC matrix, i.e., developmental trajectories and environmental/contextual influences, should also be considered.

Altered brain systems in ASD are not static [109] and are highly age dependent [1, 110, 111]; atypical cortical development in ASD also occurs across different stages, e.g., different patterns of ASD-related WM development between infancy [112] and adolescence [45]. It is noteworthy that a substantial proportion of the study cohorts reviewed in this chapter spans a wide age range, obfuscating age-related effects. Further, infants (e.g., the IBIS cohort [112]), toddlers [113], and preschool-age children [114] are still underrepresented in the literature, limiting a comprehensive depiction of atypical brain development across the lifespan. Insufficient longitudinal data further constrain the inferences about 'chronogeneity' [6]. Of relevance, recent reports with cross-sectional samples have identified highly individualized GM [115] and WM [116] features in autistic individuals as deviance from age-related typically developing norm. Overall, there is no conclusive evidence to illustrate how early neurodevelopmental disturbances in a specific RDoC system may interact with other RDoC systems and influence precursors crucial for later autistic phenotypes.

Limited numbers of twin studies suggest that atypical neurodevelopmental trajectories in ASD may not only be driven by various genetic and molecular processes, but may be also modulated by environmental factors and experience-dependent mechanisms [117]. In future expositions of RDoC for framing neuroradiological evidence of ASD, these developmental and environmental influences should be integrated.

Heterogeneity

Perils of neuroimaging replications may in part arise from phenotypic heterogeneity in the autism population. For example, all foregoing age-relevant issues may in part contribute to the heterogeneous neuroimaging findings about ASD [1, 110, 111]. Besides, there is a large number of possible strata nested in autism, e.g., by sex or gender [118], medication status, psychiatric comorbidity [119], history of language development [66], intellectual abilities alongside current language status [65], to name a few. A latest report based on one of the largest samples (~1300 participants) suggests that the patterns of altered brain anatomy in ASD closely pertain to specific sources of heterogeneity, including sex/gender, intellectual levels, and age [83]. Taking sex/gender as an example, the effects of ASD diagnosis are not consistent across males and females; there are sex/gender-by-diagnosis interactions noted across imaging modalities [118]. Systematic over-enrichment of autistic males over females and heterogeneous sex/gender ratios in study samples may contribute to the inconsistent findings.

Fundamentally, large-sample-size studies that acknowledge phenotypic heterogeneity in the autism population may be a plausible remedy to reduce biases of varied estimated sample statistics due to sampling variability [6]. Specifically, large sample sizes (at least $N > 100$ per group) have been shown to attenuate the inflation in effect size estimation. This is a particularly pronounced problem in small sample-size studies, as they are much more prone to biases of enrichment of specific strata over others [6]. Nonetheless, there are very limited homogenized large-sample single-site data or multi-

site collaborative endeavors [42, 53] data amounting to such large sample sizes. Hence, multi-site consortia such as the ABIDE [54, 55], EU-AIMS/AIMS-2-TRIALS Longitudinal European Autism Project (LEAP) [120], and ENIGMA [37] offer an unprecedented access to large datasets for the investigation of autistic brain, despite a potential constraint on the effect size estimation operating from one site to another due to heterogeneous data quality. These initiatives allow for approaches which yield discovery findings that could be replicated in an independent set, e.g., [57, 121]. Based on such large and open datasets, attempts at stratification and/or dimensional biotyping markers (unsupervised learning which searches for meaningful substructures within the ASD phenotype with different sources of imaging information [116, 121]), or implementation of the advanced computational modeling [122], might benefit personalized predictions.

Beyond the case–control design or framework, considering that ASD emerges from heterogeneous etiologies [15], it might be speculated that each autistic individual represents an exemplification of a rare disease model conforming to equifinality (by the framework of developmental psychopathology) [123], making generalizable models hardly tangible. This hypothesis is largely supported by a study examining the neuroanatomical phenotypes of 26 mouse models related to autism [124], which recapitulates that disparate etiologies contribute to heterogeneity in brain volumes. Correspondingly, these findings from mouse models may echo the fact that autistic individuals with different sets of genetic alterations have similar behavioral phenotypes, while presents with disparate brain phenotypes.

Understudied Populations

Another critical caveat is that almost all published neuroimaging evidence (>99%) [65] derives from intellectually and linguistically capable autistic populations, who represent only part of the spectrum. Those with minimally verbal expressions, intellectual disabilities, or/and developmental regression (i.e., loss of language or other adaptive skills after a period of typical development) are understudied, because they are more difficult to be evaluated using standard assessment tools or neuroimaging experiments. The paucity of data on these sub-populations undoubtedly biases full disambiguation of neurobiological accounts of ASD. Hence, future research needs to incorporate understudied population-informed experimental designs, such as extensive planning [125] and novel paradigms (e.g., sleep [80] or naturalistic movie viewing [126]).

Imaging Methodology

In-scanner head motion commonly contributes to artifactual influences on MRI studies, especially in neurodevelopmental conditions, as they tend to have higher difficulties conforming to requirement during image acquisition. While awareness of impacts of motion artifacts on the fMRI connectivity and task-evoked activity studies has recently raised [127], only a handful diffusion-weighted imaging studies to date have explicitly assured that the ASD and comparison groups did not differ significantly in head motion levels [3, 128]. Even further, objective and valid measures of sMRI data quality regarding motion artifacts have only been available recently [129]. As motion artifacts introduce spurious diagnostic group differences across neuroimaging modalities [127, 128, 130, 131], this raises the concern that results of neuroimaging studies may be influenced by diagnostic group differences in head motion. Future studies should implement careful data quality control, tight motion matching, and motion-informed preprocessing steps and statistical strategies to minimize confounding impacts of head motion. Moreover, during data acquisition, new techniques should be utilized to reduce head motion, e.g., prospective real-time motion correction (in which motion is tracked and compensated for) across modalities [132–134] and modification of the scanning environment with feedback cues about head motion [135]. The development of multi-echo fMRI [136] and multi-band multi-echo fMRI [137] (using multi-echo framework to estimate BOLD and non-BOLD signals plus multi-band techniques to simultaneously enhance spatial and temporal resolutions of the fMRI data) also improves data denoising.

Inconclusive findings may also result from other methodological factors. For example, different image processing pipelines and parameterization lead to different predictive power of classifying autism versus controls based on resting-state fMRI connectivity data [138]. Different methodological approaches to resting-state fMRI can result in divergent connectivity findings in the same dataset [94]. Different sMRI preprocessing methods also result in nuanced differences in ASD-associated altered brain volume estimates [139]. Similarly, different algorithms of diffusion tractography also produce subtle inconsistency in reconstructing existing WM tracts [140]. Fundamentally, there is an urgent need for methodological innovation and systematic examination of choices of pipelines to establish consensual MRI processing pipelines. Methodological limitations in ^{1}H-MRS and PET/SPECT can be found in [4, 5].

Measuring Idiosyncrasy

Idiosyncrasy refers to behaviors or features peculiar to an individual; variability of connectivity or activity across individuals is often referred to as idiosyncratic brain organization. As inconsistency in earlier reports fails to support the long-distance underconnectivity and local overconnectivity hypothesis of ASD [55, 94, 141, 142], emerging evidence endorses an alternative view, that idiosyncrasy is a hallmark of the autistic brain. This includes idiosyncratic spatial organization in brain functional networks [142, 143] and homotopic connections [144], increased variability in dynamic functional connectivity [145], excessive neural [52] and topographical [146] variability in task-related activation, and idiosyncratic brain patterns expressed by decreased correlations across participants as a function of social comprehension impairment [147]. Idiosyncrasy also impacts functional connectome patterns in autism [143]. Idiosyncrasy may reconcile the often contradictory connectivity findings, and help unify the foregoing 'rare disease' analogy as an etiological model and omnipresent heterogeneity phenomena through a framework of supposing greater between-individual variability in autism, rather than uniform elevation or reduction [148]. This view also highlights that the development of novel neuroimaging measures and algorithms helps clarify the atypical neurophysiology of autism. Further advances in modeling [149, 150], brain dynamics estimation [151, 152], understanding processing gradients in the brain [57], in vivo measurement of nuanced cortical tissue organizations [153], and establishing links between transcriptome and morphometry [154] alongside function [81] may provide fruitful mechanistic accounts of ASD.

Searching for Biomarkers

Beyond the sole characterization of neural correlates, researchers have begun to integrate machine learning algorithms to identify objective 'biomarkers' in ASD [155, 156]. Besides the stratification approach as illustrated above, these machine-assisted techniques also use algorithms, such as support vector machine and artificial neural networks, to learn the pattern accounting for the differences between groups, and then establish a rule whereby a given observation can be classified into an existing group (i.e., pattern classification [156, 157]). Multivariate classifiers use features, such as brain connectivity [138, 158, 159] or morphology [113], to predict diagnostic categories [157]. Prospective neuroimaging work from the IBIS network provides evidence to indicate hyper-expansion of the cortical surface [160] and a specific resting-state functional connectivity pattern [161] between 6 and 12 months of age as potential biomarkers for risk progression in ASD. A series of prospective sMRI studies suggest that a feature of excessive extra-axial cerebrospinal fluid at 6 months predicts the diagnosis of autism in children with heightened familial risk [113, 162, 163]. These longitudinal studies in at-risk toddler populations may implicate future early detection markers. In the same vein, functional connectome fingerprinting and machine learning algorithms could infer single-subject prediction of autistic symptoms [12].

Although there is potential for neuroimaging-based biomarkers for ASD, classification accuracies based on cross-sectional design, despite optimized parametrization, range from 60 to 97%, dependent on sampling, features, noises, and cross-validation algorithms [155, 156]. Thus, we have not reached the point where brain-based biomarkers could be used to clinically diagnose autism.

Conclusion

Structural and functional neuroimaging studies across modalities show that ASD is associated categorically or dimensionally, or both, with altered structures, activities, and connectivity in multiple RDoC-based systems, which contribute to brain organizations that may be suboptimal for complex information processing underpinning social-communication and executive control processes, and, potentially, superior for specific lower-level sensory-perceptual processing. Biological and psychological interventions may assuage these alterations at the brain level, reflecting neuroplasticity. However, the patterns of atypicality are not consistent across the autism spectrum. Some report purely decreased activity, structural morphometric information, or functional and structural connectivity in brain systems implicated in specific functions (e.g., a 'social brain' network [94]), others report likely compensatory phenomenon via the recruitment of uncanonical brain circuitries [70], and some others report increased brain idiosyncrasy [144]. These heterogeneous manifestations in neuroimaging findings may come from varied neurodevelopmental processes, demographics, behavioral and cognitive presentations, and imaging methodological caveats. The development of new imaging sequences, computational methods, conceptual frameworks, integration with genetics and animal model studies, and moving beyond a case–control design, are key opportunities to elucidate the heterogeneous neurobiologies of autism in the era of big and open data. Utilizing the RDoC as an organizing framework for neuroimaging/neuroradiological research has great potential to better synthesize theoretical models of autism, and to link imaging discovery to multi-level presentations, especially molecular neurobiology. These attempts may further facilitate discovery of

experimental therapeutics. Based on the RDoC framework, transdiagnostic approaches also provide complimentary information.

Multiple Choice Questions

- Which of the following systems is not included in the RDoC framework? (A) Communication Systems, (B) Negative Valence Systems, (C) Positive Valence Systems, (D) Cognitive Systems, (E) Sensorimotor Systems.
  Ans: (A)
- Which of the following factors may contribute to the heterogeneous neuroimaging/neuroradiological findings associated with ASD? (A) Sex and gender, (B) Developmental stages, (C) Co-occurring psychiatric conditions, (D) Current language level, (E) All of the above.
  Ans: (E)
- Which statement is most likely incorrect? (A) Motion artifacts in the neuroimaging data introduce spurious diagnostic group differences across MRI modalities, (B) Different image processing pipelines and parameterization will not lead to different predictive power of classifying ASD versus typically developing controls based on fMRI data, (C) Impaired motor coordination may impact both social functioning and RRBIs in ASD, (D) Structural and functional constituents of the 'social brain' largely overlap with the default-mode network.
  Ans: (B)
- What new development may help clarify the heterogeneous neurobiologies of ASD in the era of big and open data? (A) Imaging acquisition sequences, (B) Computational methods, (C) Conceptual frameworks, (D) Integration with genetics and animal model studies, (E) Moving beyond a case–control study design, (F) All of the above.
  Ans: (F)

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Neuropathology of Autism

17

Yarden Kezerle

Learning Objectives

- To realize the multifactorial nature of ASD.
- Understand how the information on the neuropathology of ASD is gathered and its limitations.
- Know the most relevant morphologic changes found so far in ASD human subjects.
- Link the knowledge brought to us by Genetics and the use of animal models with morphologic changes in human beings.

Highlights

- Morphologic alterations were consistently seen in ASD patients and its limited evidence.
- Immunologic-driven and blood–brain barrier morphologic alterations were seen in ASD.
- Heritable genetic alterations and its relationship to morphologic alterations.
- Main animal models used in ASD research.

Introduction

Understanding the neuropathological basis of such a diverse spectrum of behaviorally defined diseases like autism spectrum disorder (ASD) is a complex task for many different reasons.

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There is not much dispute over the fact that the ASD is composed of a group of conditions with the main pathophysiologic process based in the brain, and the most obvious problem from the point view of a pathologist is the difficult access to tissue, which depends virtually entirely on postmortem tissue donation. This problem has been accessed through tissue donation programs like the Autism Tissue Project (autismtissueprogram.org), autism BrainNet, and SPARK (sparkforautism.org), which try to centralize tissue donation and make it available in a more organized systematic fashion, with the goals of increasing the number of subjects and making comparisons more consistent and reliable.

Another cause of discomfort added to the task of understanding the morphologic changes and the pathologic basis of the ASD is the very diverse and florid nature of this spectrum of diseases, making some comparisons between studies very problematic; especially when one takes into consideration the differences between the diagnostic definitions of ASD through time [1]. The first reviews about the neuropathology of autism included a small number of subjects with many histological findings that may be attributed to a dubious diagnosis; sometimes the result of searches for cases of childhood psychosis or schizophrenia [2]. However, those pioneer works already made it clear that one should not expect a one-pathway, simple explanation for the neuropathology of ASD.

Given this paucity of material and the lack of consistency, the role of animal models to better understand the ASD pathologic basis became central, with diverse monogenic and copy number variation models that are well established today, with many potential risk genes being identified.

Our understanding of the neuropathology of ASD has been widened also by more recent contributions of molecular pathology and faster and cheaper sequencing technologies, creating an intersecting area of interest for pathologists, geneticists, and neurologists.

In this chapter, we are going to review what are the consistently noticed morphologic alterations in ASD so far, and where we are going from here in the comprehension of the

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ASD physiopathology. Animal models of ASD are briefly discussed in this chapter and in more details elsewhere in the book.

Whole Brain Alterations

Starting from basic measurements, the brain size and the head circumference were found to be increased when compared to age values normal, at least in a significant subset of patients [3].

Dysplasia is described in the cortex of ASD subjects in some works [4–6], sometimes concomitant with heterotopia [6], suggesting alterations in neuronal maturation and migration. In the context of Neuropathology, dysplasia must be taken in its original meaning of abnormal growth or development of cells, not implying a neoplastic nature as the term usually does for pathologists.

Macrocephaly is described in the context of some specific mutations, as in CDH8 [7]. Persistent vascular remodeling beyond childhood was noticed in several brain regions [8]. An increase in global neurons number is found in some studies, but in some, the increase in brain size can be attributed to increased neuropil. Increase in the microglia density was described throughout the brain, but some studies suggest that this alteration may be region specific [9, 10].

In the subsequent sections, we will describe the specific changes described by brain region.

Alterations Consistently Found by Region

The examination of histologic sections adds to the knowledge brought to us by neuroimaging more subtle information, like morphology of individual neuronal, glial, or other cell types, migration processes, differentiation path, and others.

Since the classic studies were started by Bauman and Kemper [11] in the 1980s, many of the alterations described proved non-reproducible, such as size and density of some specific neuronal populations [12], which may be attributable to a lack of uniformity in the diagnosis or a myriad of confounding factors that were not completely taken into account. Nevertheless, much has been added to our knowledge since then.

Cortical Alterations

One of the consistent alterations seen in ASD subjects is the increase in von Economo neuron density increase seen in the fronto-insular cortex [13, 14]. Children with ASD were shown to have an elevated ratio between von Economo neurons and pyramidal neurons in layer V of this region [15]. Given the relationship of this brain region with social interaction abilities, it is not surprising that these alterations have been found consistently in ASD subjects. A functional partner to the fronto-insular cortex, the anterior cingulate cortex was demonstrated to have poor, less distinct lamination of its layers [16]. Children with ASD show a correlation between the severity of ASD symptoms and the number of pyramidal neurons seen in the anterior midcingulate cortex [17].

Another region that consistently shows alterations in ASD is the prefrontal cortex (PFC), which is not surprising, given this region's role in cognitive control and coordination of behavior with other areas. Studies regularly show an increase in neuron number in ASD subjects compared to normal controls, with no increase of glial cells number [18].

Smaller pyramidal neurons are seen in the PFC of ASD patients, with a normal number of neurons [19, 20]. A recent study of the lateral PFC showed significant changes in the structure and organization of myelinated axons in its layer 1 in individuals with autism ([7], picture 1). A significant decrease in the number of a GABA-ergic interneuron subtype, the chandelier cell, was seen in the prefrontal cortex of subjects with autism [21].

Another cortical region involved in ASD is the fusiform gyrus, well known for its role in facial processing. Many studies show that the fusiform gyrus is hypoactivated in ASD [22–24], with one study suggesting that this hypoactivation may be the result of an altered or reduced mitochondrial metabolism [24]. This region also shows a significantly lower total neuronal density in the layers II, V, and VI and smaller perikaryon in layers V and VI (Fig. 17.1). A reduced connectivity between the fusiform gyrus and the areas responsible for facial expression evaluation is also described [25–27]. It is important to highlight that the alterations in the fusiform gyrus were evaluated in a wide age range, and more age-specific studies regarding this region are yet to be done.

In the hippocampus, smaller than normal neurons may be observed, together with an increase in cell density and poorly developed dendritic arborization [16, 28]. It is worth remembering that the hippocampus is divided into four regions (CA1 to CA4), and the dentate gyrus. The CA1 region shows focal thickening with an increase in pyramidal neuron numbers. Increased density of GABA-ergic neurons occurs in the anterior body of the hippocampus [29]. All the hippocampus CA regions show swollen axon terminals.

Cerebellar Alterations

Cerebellar involvement in emotional processing has come to greater attention, and its involvement in the pathology of ASD has been well studied, despite with some controversial results. Lesions in the cerebellum are knowingly related to

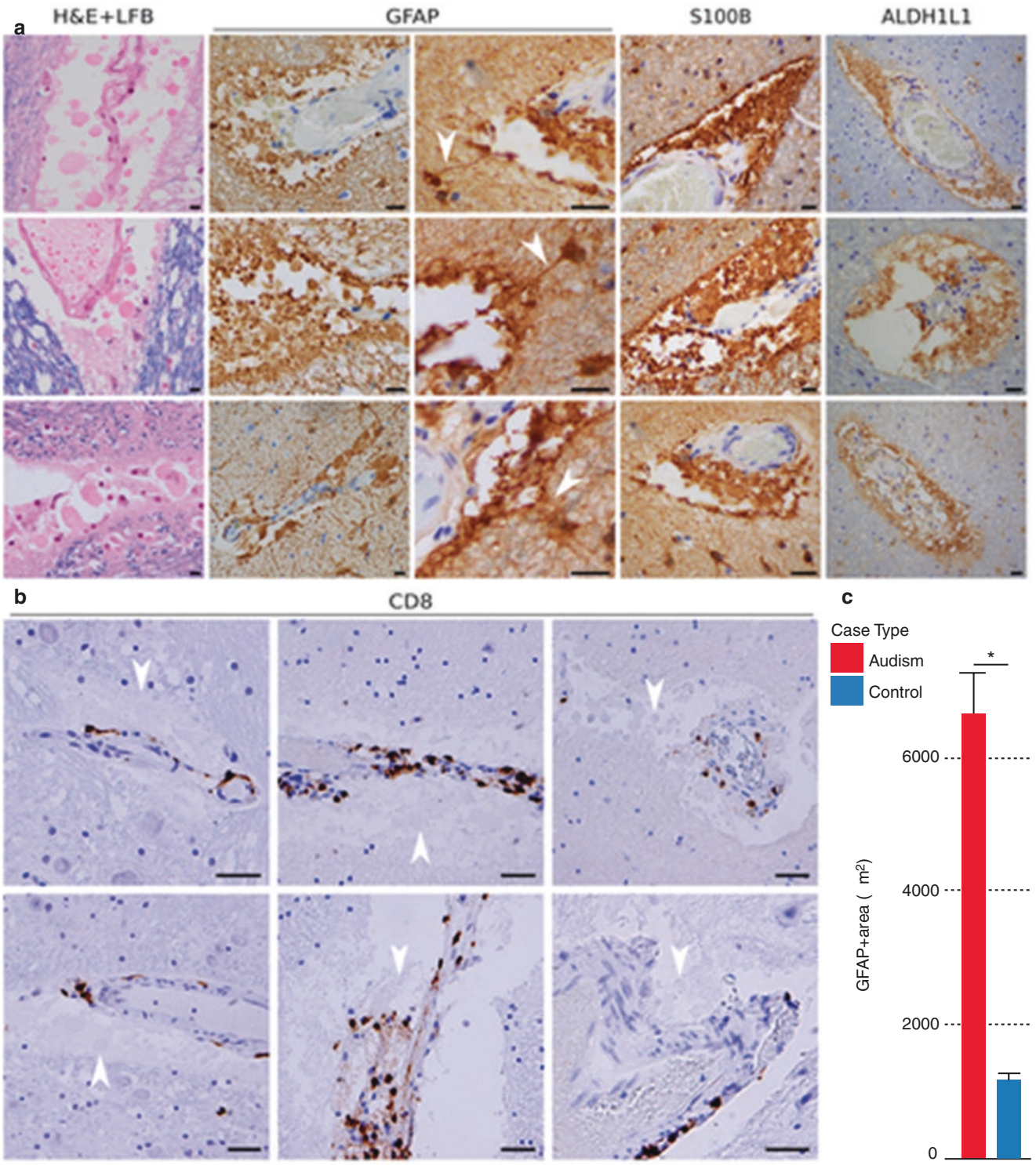

Fig. 17.1 Perivascular membranous blebs stain with multiple astrocyte markers and co-localize with CD8+ T-cells in ASD brains. Round and uniformly eosinophilic membranous blebs of varying sizes were identified in the perivascular Virchow-Robin spaces of autism brains (white matter examples, **a**; all scale bars, 40 μm). The membranous blebs are eosinophilic on H&E + LFB (**a**, column 1). GFAP, S100, and ALDHL1 immunohistochemical staining (**a**, columns 2–4) establish the blebs as derived from astrocytes. Photomicrographs taken from gray and white matter samples from ASD brain cases double stained by immunohistochemistry for CD8 and hematoxylin reveal the cytotoxic $CD8^+$ T-cells (brown) in close proximity to membranous blebs (**b**; pale blue, white arrowheads; all scale bars, 40 μm). Perivascular GFAP+ material was increased in autism compared to controls (**c**, $N_{vessels}$autism] = 209, $N_{vessels}$[control] = 120). Bar heights represent means and whiskers represent s.e.m. $^*p < 0.05$, Welch's *t*-test (extracted from reference [41]). Ann Neurol. Author manuscript; available in PMC 2020 May 9. Published in final edited form as: Ann Neurol. 2019 Dec; 86(6): 885–898. Published online 2019 Nov 4. doi:10.1002/ana.25610

depression, anxiety, bipolar disorder, panic disorder, attention-deficit hyperactivity disorder, and ASD [30].

Several studies show a diminished number of Purkinje cells in the cerebellum of ASD subjects [12, 31–33]. The main size of the Purkinje cells is also significantly smaller in these patients. Interestingly, one study showed that in ASD subjects, the loss of Purkinje cells can be interpreted as a neurodevelopmental process, with loss of cells probably occurring between 32 weeks of gestation and the immediate postnatal period [34]. Dysplasia was a consistent finding in diverse cerebellar regions [6, 33, 35, 36].

Amygdala Alterations

Another area of interest in the brain of ASD subjects is the amygdala, especially for its social behavioral role. Contrasting results can be found in the literature involving the amygdala in ASD, with older studies [11, 16] showing decreased neuron size and density, and other studies [19, 37] showing a reduction in neuron density only. Different populations of ASD subjects and methodologies may account for the different results.

Brainstem Alterations

Since Rodier and collaborators in 1996 performed the study [38] of a 21-year-old patient with ASD showing severe changes in the brainstem, with an absence of facial nerve and superior olivary nuclei, others have failed to show the same alterations [39]. Likewise, the locus coeruleus of some few ASD subjects showed dispersed nuclei and loosely grouped neurons [40], findings that other studies failed to reproduce [39].

Immunologic-Driven Alterations

Alterations of the blood–brain barrier and its relationship with the presence of activated inflammatory cells in the CNS have been studied, with round and uniformly eosinophilic membranous blebs found in the perivascular Virchow–Robin CSF spaces of some of the small caliber blood vessels in the brain from some individuals with ASD that was absent from the controls. These membranous blebs were often in proximity to CD8+ T cytotoxic lymphocytes, which are quantitatively increased in ASD subjects when compared to controls. The blebs stained positive for astrocyte markers like GFAP, S100B, and ALDH1L1 (Fig. 17.1) [41]. In the same study, adventitial perivascular collagen was increased at these vessels from ASD subjects relative to controls.

Animal Models of ASD and Related Neuropathological Alteration

A Short Briefing on Genetics

ASD is well established as a heritable psychiatric disorder in up to 50% of the cases [42–45]. These heritable patterns help establish several proteins with its expression altered or silenced leading to specific alterations in the formation and maintenance of synapses as well as in chromatin remodeling [43, 46–48]. The establishment of relationships between genetic patterns and its molecular consequences and possible causality has been accelerated in recent years through studies using next generation sequencing [46–48].

These genetic patterns are used to create animal models based on lineages of animals with specific gene ablations that produce specific symptoms that are part of the ASD. By simulating the impairments seen in ASD patients, these models are an excellent way to gather knowledge about specific neural systems affected in the presence of specific genetic alterations. This can, in turn, lead to a better understand of the many different pathologic processes that can be seen in the ASD and even gives us a prospect of a future subphenotyping of these patients using fast gene sequencing to better tailor the subsequent treatment choices.

In the next sections, we are going to review the findings that brought some light into the understanding of the ASD physiopathology, starting by animal models based on single 'ASD genes.'

Fragile X Mental Retardation Gene (FMR1)

The fragile X syndrome (FXS) has a well-established cause, being it in most cases the expansion of cytosine–guanine–guanine trinucleotide sequences [49], leading to a transcriptional silencing and the absence of the fragile X mental retardation protein (FMRP). This protein is involved in regulation of other proteins transcription, being those proteins necessary to proper synaptic plasticity [49–51].

The fragile X mutation is the most common single genetic cause of autism, occurring in 1–6% of boys with ASD [51, 52].

Although FXS is not characterized by gross brain defects, a consistent phenotype is an increased spine density and an altered ratio of mature and immature spines [50]. Studies with mice knock-outed for FMR1 showed increased spine length and density, with variations between brain areas and age groups [53, 54]. These findings are corroborated by postmortem findings [53, 54]. Alteration in spines was described in the visual and somatosensorial cortices [21, 53], hippocampus [55], nucleus accumbens [56], and dentate

gyrus [53], with different findings being seen in the same areas in different age groups [50].

Other alterations involving synaptic ultrastructure were observed, including increase in the postsynaptic density in the nucleus accumbens [56].

Tuberous Sclerosis complex1/2 Genes (TSC1/2)

Tuberous sclerosis complex is an autosomal-dominant genetic disorder, caused by mutations either in the TSC1 gene or the TSC2 gene [57, 58], leading to a loss of normal expression of the proteins hamartin and tuberin, respectively. These proteins play a role in the inhibition of the mammalian target of rapamycin (mTOR) protein. The mTOR pathway is responsible for protein translation, cell-cycle progression, and response to hypoxia. Dysregulations in the mTOR signaling not only are a component of several different neoplasms, but also cause intellectual disability, seizures, and ASD.

ASD is common in children with tuberous sclerosis and as many as 9% of all children with autism may have tuberous sclerosis [59, 60].

A model of TSC1 knockout done specifically in Purkinje cells showed postnatal loss of these cells, dendritic spines density elevation, and increased soma size in neurons [61]. Knockout of TSC1 resulted in increased spine density in basal dendrites of the temporal cortex neurons [7]. A reduction in Purkinje cells number is also seen in TSC2 knockout mice [17].

Methyl-CpG-Binding Protein 2 (MECP2)

Rett syndrome, an X-linked neurodevelopmental disorder that occurs almost exclusively in females, is mainly caused by mutations in the MECP2 gene. The loss of speech, stereotypic-hand movements, gait abnormalities, deceleration of head growth, seizures, breathing abnormalities, and ASD are features of this syndrome [62].

Animals deficient in MECP2 show reduced brain weight, thinning of the neocortex, increased cell density in the neocortex, olfactory bulb, hippocampus, and cerebellum [63–66] The layers II and III of the neocortex, layer V of the motor, and somatosensory cortices, CA1 and CA2 regions of the hippocampus, the locus coeruleus and cerebellum all showed increased cell density [51, 64–66], as well reduced spine density in the somatosensory, primary motor, and prefrontal cortices, CA1 region of the hippocampus, dentate gyrus, and cerebellum [30, 64].

Overexpression of MECP2 in mice leads to an increase in dendritic length and complexity and increased glutamate receptor density [67].

SH3 and Multiple Ankyrin Repeat Domains 3 Gene (SHANK3)

Mutations in SHANK3 are described in microdeletion syndromes as Phelan-McDermid, characterized by global developmental delay, intellectual disability, delayed speech, minor dysmorphic features, and ASD [37]. There are different isoforms of SHANK3, with variable expression by region and age, but the analysis of animal models with different mutations in SHANK3 shows consistently a deficit in glutamate-dependent neurotransmission [68, 69].

Neurexins (NRXN)

The NRXN are presynaptic adhesion molecules involved in differentiation of glutamatergic and GABA-ergic synapses, forming a complex with postsynaptic neuroligins ([67, 70, 71], see Sect. Neuroligins (NLGN)). Mutations in the genes NRXN1 and NRXN3 are known to be associated with ASD, with a reduction in the number of inhibitory synapses in the neocortex and brainstem of mice knockout for those genes compared to a wild-type control group [72].

Neuroligins (NLGN)

The NLGN are the postsynaptic counterpart of the NRXN, and mutations in the genes NLGN3 and NLGN4 are associated with ASD [55, 70, 71]. Models of NLGN3 and NLGN4 knock out animals and those of NLGN3 knock in mice, with the introduction of a mutation knowingly associated with ASD. The alterations found in these animals were reduced volume of the hippocampus, thalamus, striatum, cerebral peduncle, corpus callosum, fimbria, and internal capsule [73]. Animals with mutations in the NLGN3 gene show enhanced inhibitory transmission, caused by an increase in the strength of inhibitory synapses, keeping its number unaltered [74]. More recently a study using a mouse model expressing the R451C autism-linked mutation in NLGN3 showed that this mutation elicits the unfolded protein response in vivo, which appears to trigger alterations of synaptic function in the cerebellum [55].

Chromodomain Helicase DNA-Binding Protein 8 (CHD8)

Encoding chromodomain helicase DNA-binding protein 8, CHD8 acts as a transcriptional repressor by remodeling chromatin structure and recruiting histone H1 to target genes [63]. CHD8 haploinsufficiency is a highly penetrant risk factor for ASD, with disease pathogenesis probably resulting

from a delay in neurodevelopment [75]. Morphologic changes found in animal models include macrocephaly, reduced neural proliferation, dendritic arborization, and spine density in neurons of the layers II and III [76].

Synaptic GTPase-Activating Protein 1 (SynGAP1)

Fifty percent of the patients with SynGAP1 mutations are diagnosed with ASD [28]. Animal models suggest that SynGAP1-inactivating mutations, when present during early development, have the net effect of de-repressing the maturation of dendritic spine synapse in the hippocampus [77].

AT-Rich Interactive Domain Containing Protein 1B (ARID1B)

The gene ARID1B encodes a chromatin remodeling factor. ARID1B heterozygous knockout mice exhibited ASD-like traits related to social behavior, anxiety, and perseveration [78]. ARID1B heterozygous mice showed fewer cortical GABA-ergic interneurons and reduced proliferation of interneuron progenitors in the ganglionic eminence. ARID1B haploinsufficiency also led to an imbalance between excitatory and inhibitory synapses in the cerebral cortex [79]. A recent study showed that ARID1B haploinsufficiency in parvalbumin interneurons contributed to social and emotional impairments, while the gene deletion in the somatostatin interneurons caused stereotypies as well as learning and memory dysfunction [80].

Glutamate Receptor Ionotropic, NMDA 2B (GRIN2B)

GRIN2B haploinsufficiency has been associated with ASD [54]. GRIN2B knockout leads to a delay in the migration of cortical neurons and an increase in dendritic length and branching. The study of these models is leading to a new possibility of treatment. One study recently showed that positive allosteric modulators that target NMDA receptors in loss-of-function GRIN variants associated with neurological and neuropsychiatric disorders, including ASD, led to the partial restoration of some aspect of the reduced function [54].

T-Brain-1 (TBR1)

TBR1, a T-box transcription factor expressed in the cerebral cortex, regulates the expression of several candidate genes for ASD [81]. Mice carrying the TBR1-K228E de novo mutation identified in human ASD were identified with various ASD-related phenotypes, with the neocortex displaying an abnormal distribution of parvalbumin-positive interneurons, with a lower density in superficial layers but a higher density in deep layers [82].

Final Considerations

As one can infer from this chapter, much of the information gathered about the neuropathology of ASD waits for replication and validation, but progress has been done in that direction. No one expects to rely on histology sections to guide the management of ASD patients, but the study of these specimens and its correlation to the genetics and animal models are crucial to better develop the still fragmented knowledge that we have in the process of the ASD phenotype formation in the CNS.

Multiple Choice Questions

1. A child diagnosed as ASD with a known CDH8 gene mutation most probably will show:
   (A) Microcephaly
   (B) Macrocephaly
   (C) Normal brain volume with increased neuronal density
   (D) Normal brain volume and normal neuronal density
   CORRECT: B
2. What morphologic changes between the listed below can be consistently found in the hippocampus of ASD subjects?
   (A) Smaller than normal neurons, with an unchanged cell density and dendritic arborization
   (B) Lymphocytic infiltration and larger than normal neurons
   (C) Decrease in pyramidal neurons number in the CA1 region
   (D) smaller than normal neurons, together with an increase in cell density and a poor developed dendritic arborization
   CORRECT: D
3. Which cell type is the main known responsible for the immunologic pathogenesis of ASD?
   (A) CD20+ B lymphocytes
   (B) CD3+ CD8+ cytotoxic T lymphocytes
   (C) CD3 + CD4+ Th17 lymphocytes
   (D) IgG4-producing plasma cells
   CORRECT: B
4. In a postmortem exam of a 37-year-old man, the pathologist would expect to find in the patient's cerebellum:
   (A) Diminished number of Purkinje cells
   (B) Absence of vermis
   (C) No abnormality whatsoever
   (D) Diminished number of Purkinje cells and absence of dysplasia
   CORRECT: A

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Genetics and Epigenetics of ASD

18

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Learning Objectives

1. To become familiar with the various genetic and epigenetic contributions to autism spectrum disorder (ASD).
2. To appreciate how technological advances have deepened our understanding of ASD's (epi)genomic architecture.
3. To grasp the concept of common and rare genetic risk variants in relation to ASD's etiology.
4. To integrate the implicated risk genes and loci in the context of molecular pathways.
5. To understand how epigenetic regulation influences ASD risk genes and loci.
6. To realize the extent of environmental effects on ASD.

Highlights

- There is a wealth of literature that provides evidence for the heritable nature of autism Spectrum Disorder and is present in over 65% of cases.
- To date upwards of 100 genes or loci have been associated with a risk for ASD; however, no single gene or locus accounts for more than 1% of ASD patients.
- Several environmental factors have been shown to be associated with ASD, including parental age, gut microbiome, sex-specific traits, and the maternal gestational or intra-uterine environment.
- Epigenetic regulation bridges the effects of these environmental factors.

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Introduction

Autism spectrum disorder (ASD) is a childhood-onset, lifelong neurodevelopmental condition, characterized by impaired social communication and repetitive, restrictive behaviors. It has a worldwide population prevalence of around 1%, where recent estimates place ASD occurrence at 1 in 59 children [1]. ASD is both genetically and clinically heterogeneous, such that the affected individuals may display comorbidities, including intellectual disability (ID), epilepsy, and attention-deficit hyperactivity disorder (ADHD). Due to the widespread availability of genetic technologies, especially high-throughput microarray and sequencing platforms, the past few decades have seen significant advances in our understanding of ASD's (epi) genomic architecture. To date, both genetic and epigenetic influences have been implicated in ASD etiology.

The focus of this chapter is to illustrate the role of genetics, epigenetics, and environmental influences in ASD etiology (Fig. 18.1), and how these findings have contributed to our biological understanding of this common yet complex neurodevelopmental disorder.

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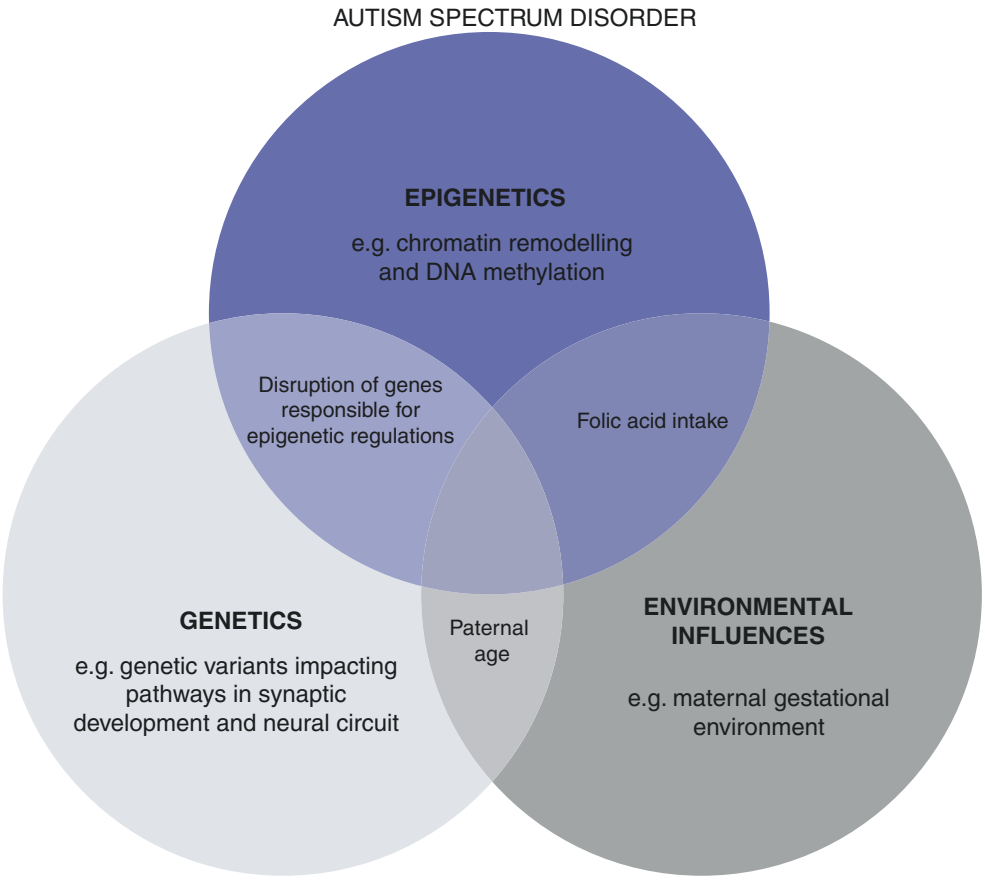

Fig. 18.1 Genetic, epigenetic, and environmental influences in Autism spectrum disorder (ASD). Genetic, epigenetic, and environmental factors have been implicated in ASD etiology. These include genetic variants, candidate pathways, epigenetic mechanisms, and environmental influences. They are often overlapping each other (e.g., paternal age and folic acid intake) to contribute to ASD risk

Genetic Etiology

Genetic Heritability of ASD

In the late 1970s, epidemiological studies of twins, siblings ('sib-pair'), and families provided the initial support for the genetic basis of ASD. Folstein & Rutter (1977) were the first to report a higher concordance rate of ASD in monozygotic twins with infantile ASD – that is, both individuals in a monozygotic twin pair displayed ASD traits (36%) when compared to dizygotic twins (0%), as well as an ~5% increase in ASD risk for siblings of ASD **probands** (i.e., the recurrence rate) when compared to the general population [2]. It should be noted that ASD diagnostic criteria have changed over the years, which may have affected the findings of early ASD studies [refer to **Chap. 14: Diagnosis** for details]. Specifically, recent epidemiological studies have reported that in fact, the sibling recurrence rate ranges from 9% to ~25%, while ASD concordance rates can reach up to 60–90% and 0–30% in monozygotic and dizygotic twin pairs, respectively [3–6]. These studies—especially the differences in the monozygotic and dizygotic twin concordance rates—strongly point to the role of genetics in ASD.

Overall, while observations from such epidemiological studies propose that ASD has high **heritability**, heritability estimates still range widely, from a low 38% to over 90% [5–7]. They differ largely due to study limitations, including sample ascertainment bias and a lack of standardization of ASD diagnoses across studies [5, 8]. However, the most recent study has robustly investigated heritability in ASD to be ~83%, with the remainder (17%) being attributed to environmental influences [9]. Taken together, epidemiological studies to date provide evidence for a substantial genetic contribution to ASD etiology.

Studies on probands and families with ASD have resulted in the discovery of both genetic variants and environmental risk factors contributing to ASD's etiology. Here, a variant is defined as DNA nucleotide changes which differ from a **reference genome** sequence.

On average, a human genome has over three million single-nucleotide variants (SNVs) when compared to a reference genome. Using **minor allele frequencies**, these variants may be classified into the following categories: *rare* (MAF < 0.5%), *low-frequency* (0.5% ≤ MAF ≤ 5%) and *common* (MAF > 5%) variants [10]. Around 99% of genetic variants are considered common—these are referred to as

single-nucleotide polymorphisms (SNPs). Recent studies have introduced another category, *ultra-rare variants*, which are variants reported only once (i.e., singletons) and not found in general population-based databases, such as the Genome Aggregation Database (gnomAD) [11].

Genetic variants may be inherited or occur **de novo**, where on average, individuals may carry around ~75–80 de novo variants in their genome, and only one to two will affect the coding region (i.e., the exome) [12, 13]. Variants may be a risk factor for ASD (i.e., found enriched in probands compared to the general population) or be considered pathogenic (i.e., found to contribute mechanistically to the phenotype), and thus, can be subdivided into the following categories: pathogenic, benign, or of uncertain significance [14, 15]. It should also be noted that the identification of ASD risk genes and loci through statistical approaches does not necessarily confirm their causality in disease, and further functional studies, such as pre-clinical models, are required to confirm their pathogenicity.

Genomic Architecture of ASD

ASD is often comorbid (i.e., occurs simultaneously) with other genetic syndromes. The most common monogenic disorders comorbid with ASD include

- *Fragile X Syndrome*: an **X-linked**-dominant genetic disorder resulting in various neurodevelopmental conditions (including ID and cognitive impairment), where a majority of cases are caused by the pathogenic expansion of the CGG trinucleotide repeat in *FMR1*. Up to 50–60% of individuals with Fragile X syndrome develop ASD traits, which represent only 0.5–20% of ASD cases [16].
- *Rett Syndrome*: a rare neurological disorder where patients display cognitive impairment, impaired communication, and a regression in motor skills. X-linked-dominant **loss-of-function** or gain-of-function variants in the *MECP2* have been implicated in 96% of patients with Rett syndrome—and importantly, ~61% of individuals with Rett Syndrome are also diagnosed with ASD [16, 17].
- *Tuberous Sclerosis Complex (TSC)*: an **autosomal-dominant** disorder characterized by the presence of multiple benign tumors, where pathogenic variants in *TSC1* and *TSC2* are responsible for the phenotype. 25–50% of TSC patients also meet ASD diagnostic criteria, representing only 0.4–2.9% of total ASD cases [16, 18].

While there are over 100 syndromic disorders related to ASD, these disorders collectively do not account for more than 10% of ASD cases, reiterating an earlier point: ASDs are a clinically and genetically heterogeneous group of disorders [19]. Instead, early studies employed the use of approaches, such as **linkage analysis** and **candidate gene** studies, in order to dissect this genetic heterogeneity.

Several full-genome linkage analyses have been carried out in ASD patient populations, with linkage being reported for several chromosomal markers [20]. However, there is considerable variability in consensus among linkage analyses, which is in part due to varying sample sizes and genetic heterogeneity. To address these problems, subsequent studies recruited more homogenous ASD populations and further stratified patients by phenotypic characteristics (such as language development and repetitive behaviors), resulting in certain loci being replicated, e.g., the 2q, 7q, and 17q regions [18, 21, 22].

Furthermore, candidate gene approaches have implicated ASD risk genes. For example, over 15 studies have investigated the occurrence of SNPs in the Reelin gene (*RELN*) and identified a significant association between the **refSNP** rs362691 variant and ASD risk [23]. However, similar to linkage analyses, the reproducibility of candidate gene studies remains low, with common negative and false positive results – even for genes such as *RELN* which have frequently been reported to be associated with ASD [21–23].

Overall, linkage and candidate gene analyses have implicated only a few loci and genes in ASD. This is likely due to a combination of ASD's heterogeneity, **variable expressivity**, **incomplete penetrance,** and the contribution of genetic interactions to ASD phenotypes.

The development of genome-wide SNP arrays facilitated the application of **genome-wide association studies** (GWAS) in numerous studies, allowing for the investigation of the association of common genetic variants with ASD, to potentially identify ASD susceptible genes or loci. GWAS were initially limited by sample size and consequently were unable to detect genome-wide significant loci. To overcome this limitation, large-scale international collaborations compiled ASD probands into larger cohorts for GWAS.

GWAS findings suggest that a 'common disease-common variant' hypothesis may explain ASD etiology – that common genetic variants confer a small effect individually (approximately a 1.1-fold increase in relative risk) but act additively to increase the overall ASD risk (which can range between 15–50%) [24–27]. For example, in a recent GWAS meta-analysis for over 16,000 ASD individuals, a single genome-wide significant locus was found at 10q24.32 (an intronic marker), along with variants of 'borderline' significance to ASD risk, such as those in *ASTN2*, *MACROD2,* and *HDAC4*, suggesting that such SNPs of small effects contribute to a polygenic genetic architecture [28]. In fact, the idea of a polygenic architecture has prompted **polygenic risk score** (PRS) analyses to gain traction recently as a method to explore an association between a trait (in this case, clinical

characteristics of individuals with ASD) and the combined effects of SNPs [29]. Initial PRS studies demonstrated an association between ASD risk and polygenic influences – that polygenic variation contributes additively to risk in individuals with ASD who carry a de novo variant [30, 31].

While few common variants conferring ASD genetic risk have been identified so far, there is instead substantial evidence pointing to the role of rare genetic risk variants to ASD susceptibility. A 'common disease-rare variant' hypothesis has also been proposed, where it is believed that rare but highly penetrant variants (i.e., have a high probability of effect on phenotype) contribute significantly to ASD.

Approaches such as **karyotyping** and **fluorescent in situ hybridization** were the first to detect various chromosomal abnormalities (>3–5 million base pairs) in ASD probands, including the 15q11–13 loci as well as sex chromosome aneuploidies [32, 33]. In total, karyotypes identified chromosomal abnormalities in 2–5% of ASD cases [18, 34].

The subsequent development of **hybridization-based microarray approaches** enabled researchers to resolve chromosomal abnormalities down to a kilobase (kb) level. These smaller genetic imbalances are referred to as copy-number variants (CNVs): DNA segments of at least 1 kb in size which differ in copy number compared to a reference genome [35, 36].

Initial microarray approaches found that both rare de novo and inherited CNVs contributed to ASD's genetic etiology. Studies showed that de novo CNVs occur more frequently (5–15%) in ASD patients, than in unrelated controls (1–2%) or parents and unaffected siblings, suggesting that de novo variants may be a significant source of ASD risk [37, 38]. Subsequent studies found that de novo CNVs contributed to ASD risk in over 10% of simplex cases, with a greater number of highly penetrant CNVs being found in female ASD patients [37, 39].

Most ASD-related CNVs were found to be rare or unique events. However, certain loci were found to occur recurrently, including deletions at the 1q21.1, 15q11.2–13.3, 16p11.2, and 22q11.2 loci and duplications at the 7q11.23, 15q11.2-q13.1, and 16p11.2 loci [40]. **Dosage sensitivity** was observed at various loci, such as the 16p11.2 locus, where both micro-deletions and duplications are found to result in ~1% of ASD cases [41, 42]. It should also be noted that CNVs are not limited to the exome. For example, intragenic rearrangements within *NRXN1* have been reported for three ASD families, and rare inherited CNVs have been reported to affect non-genic intervals in ASD cases, which includes loci such as 16q21 and 2p16.3 [43, 44].

Of note, studies found that ASD probands had a greater burden for large CNVs, compared to controls, and that ~5% of ASD individuals carried two or more penetrant variants [45, 46]. Findings such as these have led to the proposal of a 'threshold' model for ASD pathogenicity, where different CNVs are believed to have different penetrance levels, modulated by the function of the genes impacted and dosage sensitivity [47]. While some CNVs will have a large impact (especially those which are de novo), other CNVs have moderate or little effect and only cross the ASD phenotype 'threshold' in combination with other genetic (or non-genetic) factors (i.e., 'multiple hits') [48–50]. For example, in a study of 2312 children carrying a CNV associated with ID and chromosomal abnormalities, an additional large CNV was identified in 10% of patients, which in combination with the primary CNV, resulted in the disruption of more genes, and hence, had an additive impact on the overall severity of the neuropsychiatric phenotype [49]. Similarly, a recent study of 20 individuals with a 7q11.23 duplication (Dup7), and either comorbid ASD or no ASD, showed that in the presence of the same pathogenic Dup7 variant, rare and low-frequency genetic variants act additively to contribute to components of the overall Dup7 phenotype [51].

Overall, CNV analyses have identified multiple new ASD risk genes (including *SHANK2* and *NRXN1*) and pathways (such as chromatin regulation and synapse function) to date. Interestingly, genes and pathways are associated with loci implicated in other common neurodevelopmental disorders, such as ID, epilepsy, and schizophrenia [37, 52–54].

With the introduction of **next-generation sequencing** (NGS) technologies, including targeted gene panels, **whole-exome sequencing** (WES), and **whole-genome sequencing** (WGS), it is now possible to conduct unbiased genome analyses of ASD individuals, trios, and larger cohorts in a high-throughput manner. Today, numerous NGS studies have identified a number of highly penetrant variants in previously implicated ASD risk genes and loci. In addition, NGS approaches have been used to detect multiple variant types in ASD, including single-nucleotide variants (SNVs), CNVs, insertions/deletions (indels), and structural variants (SVs).

As NGS approaches are being used to analyze ASD genomes, it is becoming increasingly clear that similar to de novo CNVs, de novo SNVs also contribute to ASD risk, with de novo exonic variants contributing to ~10–25% of ASD cases [39, 54]. While the number of de novo variants is similar in probands and controls, ASD probands are likely to have a two to threefold increase in the number of de novo LOF variants compared to their unaffected siblings [54–57]. De novo variants tend to be paternal in origin (~4 folds higher) and correlate positively with paternal age (where every 10 years of parental age correlates with a 1.3-fold increase in the number of de novo variants) [39, 58–60]. Of note, de novo loss-of-function variants are enriched in female ASD probands [54, 57].

Importantly, de novo variants of interest are not restricted solely to the exome. Recent studies have found: (i) an enrichment of de novo variants in non-coding (15.6%) regions in ASD probands, compared to controls; (ii) an enrichment of

de novo variants in promoter regions in the central nervous system, and (iii) that 1–3% of ASD individuals, with no prior diagnostic variant identified, carry pathogenic de novo variants in regulatory regions [56, 59, 61, 62].

NGS studies also provide evidence supporting the role of inherited variants in ASD etiology. For example, studies have shown that (i) in simplex families, the rate of both rare, damaging de novo and inherited SNVs are higher in ASD probands than unaffected siblings [63, 64]; and (ii) X-linked and rare autosomal recessive variants result in a 2% and 3% increase in ASD risk, respectively [65]. Similarly, **biallelic** variants have been found in both known and novel ASD risk genes (such as *AMT, PEX7,* and *SYNE1*) for both consanguineous and non-consanguineous ASD families, confirming a role for inherited causes for ASD [66]. It should also be noted that in multiplex ASD families, affected siblings do not always carry the same rare ASD risk variant, and such siblings display greater variability in their clinical phenotype, which once again emphasizes ASD's genetic heterogeneity [67].

NGS studies have also found that rare, inherited loss-of-function SNVs are enriched in probands, compared to unaffected siblings, with a significant maternal transmission bias [68]. In contrast to this maternal bias, a recent study found that rare paternally inherited SVs disrupting cis-regulatory elements, were preferentially transmitted to affected offspring in comparison to unaffected siblings, and acted as a source of ASD risk [69]. Overall, these findings point to inherited causes underlying ASD but suggest that there is greater complexity than previously hypothesized regarding parent-of-origin effects on ASD risk [70].

Recently, **post-zygotic mutations** (PZMs) have emerged as an ASD risk factor, where they are estimated to contribute ~3–8% to ASD's overall genetic architecture [54, 71, 72]. PZMs have been reported in previously implicated ASD risk genes, including *CHD2*, *SCN2A,* and *SYNGAP1*, as well as in novel candidate risk genes [54, 71, 72]. This is an area in need of further research, as currently, there are no standardized variant calling pipelines for PZMs, and their impact on ASD has not been fully delineated [73].

Tandem repeats (TRs) expansions have also been implicated in ASD, such as the CGG trinucleotide repeat expansion in Fragile X Syndrome. Similar to PZMs, pipelines to detect repeat expansions from NGS have recently been developed, which include algorithms such as ExpansionHunter Denovo and STRetch [74–77]. Interestingly, recent studies point to TRs being a potential disease-causing mechanism in brain-related disorders often comorbid with ASD. For example, *C9orf72* hexanucleotide repeat expansions have been implicated in rare schizophrenia cases, and intronic repeat expansions have been reported in different epilepsy syndromes [78, 79]. Recently, genome-wide characteristics of TR expansions were investigated in 17,231 genomes of individuals with ASD, their families, and control individuals, demonstrating that TR expansions contribute a total of 2.6% risk to ASD [80]. By continuing to investigate TR expansions in larger cohorts, with improved algorithms, we can better understand the complex genetic etiology of ASD and associated neuropsychiatric disorders. For example, in a recent genome analysis for 259 unrelated adults with schizophrenia, TR expansions with potential clinical implications were identified in *DMPK* and *ATXN8OS* [81].

Other variant types implicated in ASD etiology include mitochondrial DNA (mtDNA) defects and non-coding RNA variants [82–84]. For example, a recent study has found that mtDNA variants occur more frequently in ASD probands compared to controls and generally occur simultaneously with other ASD-associated genetic risk variants [84]. Such variant types remain under-characterized in ASD genomic architecture, and highlight the growing need to analyze ASD cohorts using WGS to ensure all variation within the genome (especially non-coding regions) is being considered when it comes to ASD etiology. In fact, the WES diagnostic rate in children with ASD is currently ~8.4%, but with the application of WGS, the diagnostic rate rises up to 11–25% [67, 85].

In summary, ~100 genes or loci have been implicated in ASD to date, including common and rare variants, where no single gene or locus accounts for more than 1% of all ASD cases. In the early 2000s, only 2–3% of ASD cases had an identifiable genetic diagnosis, but today, a genetic etiology for up to 20–30% of ASD cases can be identified. Of note, de novo and inherited rare variants are estimated to be causal in 10–30% of ASD cases [86].

From ASD Candidate Gene to Candidate Pathways

To determine the molecular mechanisms underpinning ASD and ultimately develop pharmacological and behavioral interventions for its treatment, it is important to gain a comprehensive understanding of the molecular and neurological pathways disrupted in this disorder.

Since the identification of the genetic component of ASD, different approaches have been taken to understand the pathophysiology of ASD and delineate its underlying genetic and molecular pathways. Studies investigating the transcriptional networks of ASD individuals, and functional annotation of gene lists implicated in ASD using experimental models, such as cerebral **organoids** and rodent models, are among various approaches aiming to understand the role of the implicated genes and networks [87].

Examining the transcriptional networks and gene expression profiles during fetal and early postnatal development of brains from ASD donors shed light on the genes and path-

ways with shared neurobiological functions [88]. Consistent with clinical presentations of ASD individuals, ASD risk genes are highly expressed during cortical brain development and are specifically enriched in transcriptional modules implicated in synaptic function [88, 89]. Gene co-expression network analyses point to orchestrated regulatory networks controlling the expression of ASD risk genes in the developing human brain, dysregulations in which underlie the etiology of ASD, highlighting the pivotal role of early fetal development in ASD.

Regulation of Gene Expression Mechanisms

Examining the de novo variants identified using combined results of multiple WES studies integrated with brain gene expression profiles, revealed the enrichment of genes involved in transcription and chromatin regulation among ASD risk genes [90, 91].

Chromatin remodeling plays an essential role in transcription regulation and is a prominent pathway regulating the formation of neural connections, neurogenesis, and neuronal differentiation. The association of chromatin modifiers with ASD and other neuropsychiatric disorders indicates the pivotal role of mechanisms regulating gene transcription during brain development.

Epigenetics and Chromatin Remodeling

DNA methylation, and histone post-translational modifications (PTMs), e.g., histone methylation and histone acetylation, are among the most prominent **epigenetic** marks, which regulate the accessibility and transcription of different chromatin regions. Epigenetics play pivotal roles during development through directing processes such as cell differentiation, tissue specification, and maintenance of cell lineages [92]. Parental epigenetic marks may be inherited to the offspring, thereby regulating gene expression in a parent-of-origin-specific manner. For example, *UBE3A,* a gene located at the **imprinted** gene cluster on 15q11–13, is exclusively expressed from the maternal chromosome [93].

There is emerging evidence that environmental exposures, as well as genetic defects, contribute to disease risk in the human population. Epigenetics offer a biological mechanism linking environmental changes to gene expression alteration in the pathogenesis of disorders. Epigenetic marks regulate cellular plasticity and provide the ability of a cell to respond to its environment through the rapid regulation of cell processes such as genome stability and gene transcription [94]. Therefore, the role of epigenetic regulation may explain the incomplete penetrance and heterogeneity of some disorders, including ASD.

Importantly, epigenetic processes play a large and complex role in brain development and neurodevelopmental disorders [95]. The association of ASD with epigenetic changes is supported by multiple lines of evidence; the dysregulation of genome-wide DNA methylation in ASD individuals, in addition to the functional association of many genes with epigenetic changes, such as the chromatin remodeler *ARID1B* and *CHD8* [96].

DNA methylation is a widely studied epigenetic control and plays an important role for regulating cell differentiation. Most of the DNA methylation studies so far focus on CpG sites (cytosine nucleotide followed by a guanine nucleotide). It results in the transcriptional repression of certain regions of the chromatin which in turn alters gene transcription through impeding the binding of transcription factors, modifying the recruitment of chromatin remodelers and histone modifiers, as well as the binding of methyl-CpG-binding proteins, such as MECP2. *MECP2* encodes a methyl-CpG-binding protein, and variations in this gene cause Rett syndrome (discussed above), characterized by seizures, language dysfunction, and ASD-like behaviors [16, 17]. These changes to chromatin accessibility ultimately facilitate the formation of a compact inactive chromatin.

There is emerging evidence suggesting that DNA methylation patterns change in the autistic cerebral cortex regions [97–99]. These differentially methylated genes are implicated in multiple cellular processes including immune response, synaptic pruning, and microglial cell differentiation; processes associated with ASD [98]. Intriguingly, loci implicated in GABAergic system genes (will be discussed in Sect. “GABA- and Glutamate-Mediated Neurotransmission”), such as *GABBR1*, are among the regions that are differentially methylated in the brains of ASD-affected individuals [99].

Hydroxymethylcytosine (hmC) is another major epigenetic modification in the neuronal DNA, generated through the oxidation of methylcytosine [100, 101]. Studies in mice showed that hmC modifications are highly enriched in genic and promoter regions, but are depleted in intronic and intergenic regions, and are associated with increased transcription rate of the neuronal genes [101–103].

Comprehensive genome-wide hmC maps reveal differential hmC patterns during neurodevelopment [102, 104, 105]. Brain hmC levels are increased from early postnatal stages through adulthood, indicative of a strong correlation between neurodevelopment and hmC patterns [104, 106]. Epigenome-wide studies of hydroxymethylation show that hmC-mediated epigenetic regulation of gene expression is crucial during development as well as the adult brain homeostasis. Therefore, hmC modification may offer a potential mechanism explaining the pathogenesis of neurodevelopmental disorders [104].

Consistent with their essential role during neurodevelopment, alterations in hmC modifications are associated with ASD pathogenesis. Homozygous knockout *Cntnap2−/−* mice models featuring neuronal and behavioral ASD characteristics present genome-wide disruptions in

hydroxymethylation patterns, in particular in known ASD genes such as *NRXN1,* suggesting that differential hydroxymethylation is associated with ASD [107]. The enrichment of differentially hydroxymethylated genes in ASD-implicated pathways highlights the necessity for better understanding hmC modifications in neurodevelopmental disorders, including ASD. For further reading on DNA methylation and hydroxymethylation, please refer to [100].

Collectively, these findings reveal the pivotal role of DNA methylation and its regulators in gene transcription, and indicate their aberrations as underlying molecular pathways in ASD. It should be noted that most studies thus far have examined the methylation and expression levels of the genes in a specific region of the brain, whereas different neuronal subtypes may exhibit differential spatial and temporal expression and methylation of genes. Future studies providing a comprehensive spatiotemporal understanding of the epigenetic changes on a single-gene scale, as well as genome wide in ASD and other neurodevelopmental disorders, can therefore potentially identify novel biomarkers facilitating their molecular diagnosis.

Histone PTMs, such as acetylation and methylation, are among other epigenetic mechanisms regulating gene transcription. Histone PTMs alter the accessibility of the chromatin and facilitate the binding of the additional proteins, such as transcription factors, to induce gene transcription and downstream cellular pathways. Studies on post-mortem brain samples of ASD using chromatin immunoprecipitation sequencing investigating for active enhancers, identified a common acetylome signature at >5000 cis-regulatory elements in prefrontal and temporal cortex [108]. This differential acetylation pattern in ASD subjects suggests that alterations to histone acetylome in ASD brains may underlie the differential gene transcription regulation in ASD individuals. The acetylome aberrations highlight genes involved in synaptic transmission, ion transport, chemokinesis, histone deacetylation, and immunity, consistent with the association of these pathways with ASD pathogenesis [108]. These results suggest a role for epigenetic regulation in ASD and highlights the necessity for further studies investigating acetylation patterns during different stages of brain development.

Transcription Factors

Consistent with their essential roles during brain development, variants disrupting the genes-encoding **transcription factors** (TFs) have been detected in various neurodevelopmental disorders, including ASD. For example, *FOXP1* encodes a TF essential for neurodevelopment, where *FOXP1* variants have been identified in ASD and intellectual disability cases [109]. Mice with a brain-specific *Foxp1* deletion exhibit ASD-like behaviors and defective spatial memory, consistent with the role of *Foxp1* in brain development [110]. The homeobox transcription factor, *En2*, is another susceptibility locus for ASD, as mice and human individuals carrying mutants of this gene present altered cerebellar patterning and ASD-like characteristics [111].

Alternative Splicing

In addition to transcription regulation, mechanisms modulating protein expression following transcription are also associated with neurodevelopment. Posttranscriptional modifications, in particular **alternative splicing** (AS), play essential roles in brain development. There is growing evidence suggesting that defective AS contributes to the pathogenesis of ASD. In particular, misregulation of neuronal microexons (≤51 nt) in ASD brains indicates the importance of protein interaction networks and their remodeling during neurogenesis and brain development [112]. These neuronal microexons are regulated through the binding of neuronal-specific RNA binding proteins, such as nSR100/Srrm4, which is essential for AS [112].

Following the identification of the fundamental roles of AS-related mechanisms during brain development, efforts have been made to identify pathways regulating the biologically essential exons and introns implicated in ASD. In a study of AS pathways associated with microexons involved in ASD, genes regulating Srrm4-dependent AS in neuronal microexons were identified [113]. Interestingly, *SRSF11*, which is among the genes identified as a microexon regulator, has previously been suggested as an ASD candidate gene [46]. These studies reveal the prominent role of AS in brain development and highlight the need for further studies on AS pathways involved in ASD.

MicroRNA Gene Expression

MicroRNAs (miRNAs) regulate the expression of genes post-transcriptionally through targeting their mRNA transcripts for degradation or repressing protein translation [114]. Dysregulations in the functions of miRNAs are identified in numerous diseases including those affecting cognition and behavior.

Post-mortem brain studies have suggested differential expression of miRNAs in individuals with ASD compared to healthy controls [115]. Furthermore, variants in genes regulating miRNA transcriptions are identified in disorders with ASD traits. For example, defects in *DGCR8* expression, which functions in miRNA biogenesis and regulates synaptic plasticity in prefrontal cortex, have been identified in cases of velocardiofacial syndrome which is associated with ASD traits [116].

To date, >200 miRNAs have been associated with ASD [115]. The precise function of these miRNAs is yet to be described; nevertheless, many of these miRNAs are involved in the transcription regulation of genes implicated in neurogenesis and synaptogenesis processes [117]. Interestingly,

SHANK3 and *NRXN1* are among the targets of differentially expressed miRNAs in ASD-affected individuals. These findings propose mechanistic implications of miRNAs in the pathoetiology of ASD.

Synaptic and Signaling Pathways

An orchestrated synaptic network is essential for neural physiology and neurodevelopment. Disruption in this network is a hallmark of many neuropsychiatric and neurodevelopmental disorders, including ASD.

Synaptic Development and Altered Neural Circuit

Many ASD individuals are identified with disruptive variants in genes such as *SHANK* and the *NRXN*/*NLGN* family of genes, which encode components required for synaptic development and refinement; suggesting that disruptions in synapse development and postnatal synaptic maturation pathways may underlie abnormalities in ASD [118]. *NRXN* and *NLGN* are family of genes which encode presynaptic and postsynaptic transmembrane proteins and cell adhesion molecules and thereby promote the formation of synapses and synaptic transmission. Variants in these genes have been reported in cases of ASD, supporting that defects in synapse formation and transmission during the development of the brain contribute to ASD [119].

Neuroimaging studies of cortical and corticostriatal circuits of brains from ASD individuals revealed abnormalities in network connectivity and its resting state, and pronounced structural changes to the ASD brain, encompassing several regions including cerebellum, cerebral cortex, amygdala, and hippocampus. These structural changes vary from enlargement and hyperplasia to abrogation of the white matter and alterations in connectivity across various brain regions [120]. Neuroimaging experiments in mouse models of ASD recapitulated these findings, suggesting that alterations in brain circuitry may underlie the social behavior abnormalities in ASD [121]. Notably, *Shank3*-deficient mice which exhibit autistic-like behaviors are characterized with striatal dysfunction and reduced corticostriatal excitatory synaptic transmission [122].

GABA- and Glutamate-Mediated Neurotransmission

The imbalance between excitatory and inhibitory neurotransmitters during critical stages of development is proposed as a key mechanism underlying ASD. Several lines of evidence support the involvement of excitatory (glutamate-mediated) and inhibitory (GABA-mediated) neurotransmission as major ASD-associated signaling pathways, providing therapeutic targets for this disorder. CNVs and truncating variants in genes encoding for GABA are identified in individuals with ASD [123]. Furthermore, both post-mortem brain studies of ASD individuals and rodent ASD models exhibit defects in GABAergic and glutamate-mediated neurotransmission [124–127]. For example, *Shank3* knockout mice exhibit reduced glutamate and GABA concentrations in striatum [124]. *SHANK3* encodes a synaptic protein involved in the pathology of ASD, which signals through the neuroligin–neurexin complex. These results suggest that imbalance in glutamate and GABAergic neurotransmission contributes to the neurochemical defects observed in ASD individuals and may underlie clinical symptoms associated with ASD, including intellectual disabilities and seizures. Nevertheless, it is still unclear how synaptic inputs influence behavioral and neural circuitry abnormalities in these individuals. Further electrophysiological and behavioral studies are, therefore, required to comprehensively decipher the role of excitatory and inhibitory neurotransmission pathways in ASD.

There is growing evidence suggesting a role for multiple other signaling pathways in brain development and ASD, including the calcium and calmodulin signaling pathways, which mediate the transmission of action potentials from the presynaptic to postsynaptic neurons and regulates the release of neurotransmitters. For more details on such functional studies, refer to **Chap. 19: Pre-clinical Models.**

Environmental Factors

While genetic heterogeneity may explain a fraction of the phenotypic heterogeneity in individuals with ASD, it fails to incorporate the effect of environmental factors on these genetic variants. It is now known that genetic variants alone are not usually sufficient to completely explain a disease process, but instead they are one component of a larger complex molecular system that, as a whole, results in the observed phenotype. ASD traits too are consequences of the interplay between genetic and environmental factors [128].

ASD could be considered as a psychopathological organization resulted by the combined effects of diverse biological and/or psychological factors, including genetic factors, epigenetic and environmental factors, as well as the interaction between genes and the environmental factors (gene × environment).

To date, more than 50 prenatal factors have been studied for their association with ASD, and we know various perinatal and neonatal factors contribute to ASD risk, including neonatal infections, respiratory distress after birth, maternal gestational bleeding, and feeding difficulties (for reviews, refer to [129]).

Lack of reliable data on environmental exposures collected at the appropriate risk timing windows challenges the precise assessment of the influence of environmental exposures on health outcomes. Furthermore, the precise

mechanism through which these factors contribute to the pathogenesis of ASD is not fully described yet; however, a potential link may be through epigenetic regulation and differential methylation of ASD risk genes. For example, studies on human subjects have revealed the effect of childhood abuse on DNA methylation patterns in the brain, which in turn alter gene expression [130, 131]. In addition, it has been suggested that assisted reproductive technology (e.g., in vitro fertilization) is associated with a higher ASD risk. The proposed mechanism is through differential epigenetic regulation as a consequence of hormone exposures, freezing of the gametes, and growth conditions of the embryos (Liang et al.; 2017). Nevertheless, comprehensive investigations of larger samples are required to establish a robust association and delineate the underlying mechanisms.

Maternal Gestational Environment

Epidemiological studies have suggested an association between exposures to medications, prenatal stress, and prenatal maternal nutrition and ASD risk. Prenatal maternal stress is positively associated with the risk of ASD, as well as other neurodevelopmental disorders, such as ADHD [132].

A classic example of an environmental factor introducing epigenetic changes associated with ASD is the maternal use of valproic acid (VPA) during early pregnancy [133, 134]. VPA has been widely used as an antiepileptic drug and in bipolar disorder treatment for years [135]. Clinical studies revealed that the exposure to VPA in utero results in a syndrome with features similar to ASD and children exposed to valproate in utero have an eight fold increased risk of ASD [136]. Similarly, mice exposed to VPA exhibit ASD-like behavioral characteristics, suggesting a role for this drug in the pathology of ASD [137]. VPA exposure transiently increases acetylated histone levels in the embryonic brain through the inactivation of histone deacetylase, followed by increased rates of apoptotic cell death in the neocortex [137]. Furthermore, mice prenatally exposed to VPA show decreased expression of *Nlgn3* in hippocampal regions, disruptive variants in which are associated with ASD [124]. These findings suggest that VPA administration is associated with an increased risk of ASD.

Maternal prenatal nutrition, and in particular folate supplementation, is proposed to have a protective effect with a reduced risk of ASD in the offspring [138]. These observations are in agreement with the preventive effect of folic acid as a methyl group donor from neural tube defects by promoting normal DNA methylation patterns during development [139]. Despite the unambiguous role of folate in the neural development, it should be noted that some studies have reported an increased risk of ASD associated with high, unmetabolized folic acid levels in serum [140]. Maternal malnutrition is also associated with the increased risk of ASD, such that fiber-rich and vegetarian diets which do not provide enough Zn^{2+} intake are associated with ASD risk [141].

Parental Age

To date, more than 40 epidemiologic studies have suggested a link between the increased parental age and the risk of ASD. One such study examined the association between maternal and paternal age independently with the risk of ASD in the offspring in a cohort of >400,000 Swedish ASD-affected children, concluding that advancing parental age is associated with an increased risk of ASD in children [142]. Furthermore, the difference between maternal and paternal age also contributes to the risk of ASD [9].

Mice studies investigating behavioral and brain structural changes and their link with advanced parental age, report an increased rate of de novo variants in the offspring of older sires [143]. WES and WGS studies have confirmed the higher rate of de novo aberrations and mutations, as well as chromosomal abnormalities with increased paternal age [60, 144]. Furthermore, changes of transcriptomic and DNA methylation are reported in the children of older fathers [145]. These findings propose increased de novo variations as an underlying mechanism explaining the increased ASD risk associated with advancing parental age.

The accumulation of environmental toxins, implicated with greater risk of neurodevelopmental disorders including ASD, may suggest another mechanism for the higher risk of ASD associated with increased parental age [146]. In the future, it will be essential to conduct thorough epidemiological studies integrating genetic, epigenetic and environmental data, with the careful characterization of parents, siblings, and probands with ASD. With the advances in sequencing technologies becoming more widespread, we will be expecting more insights into the genetic and molecular mechanisms involved with ASD.

Gut Microbiota

The gut microbiome serves as a nutrient intake by providing the enzymes required for the degradation of polysaccharides. For example, the utilization of lactate in milk as an energy source is facilitated by gut microbiome [147]. Furthermore, the gut microbiome functions in the development of the immune system, such that the intestinal immune system adapts to the helpful bacteria and does not tolerate pathogenic species. Gut microbiota is also linked to the central nervous system through the so-called 'microbiome gut brain axis,' whereby gut microbiome functions in the production of chemicals essential for the brain function, such as serotonin, dopamine, and GABA, essential for pathways is implicated in synaptic function [148].

Patients with ASD manifest gastrointestinal (GI) abnormalities in addition to behavioral and social communication symptoms [149]. Several studies provided evidence that gut microbiota is altered in ASD individuals with the overrepresentation of some bacterial species [150]. ASD children with GI symptoms also exhibit abnormalities in their immune response as determined by altered levels of regulatory cytokines, providing evidence for the observed immune system defects in ASD individuals [151]. It is still unclear how GI dysbiosis contributes to the pathophysiology of ASD, and further studies are needed to investigate this link.

Sex-Specific Traits and Gene–Environment Interactions

As mentioned in Chap. 13, male individuals are more frequently affected with ASD, compared to females. However, only some of the risk genes identified in ASD are located on the sex chromosomes; therefore, it is unlikely that the male bias observed in ASD can be explained solely based on these genes. Studies on *CNTNAP2* knockout mice modeling ASD propose that genetic and environment interactions may contribute to sex-specific traits observed in ASD [152]. Examining stress-induced maternal activation of the immune system reveal that histone methylation levels are significantly affected in the left hippocampus of male *Cntnap2*-deficient mice, suggesting that maternal immune activation preferentially affects males compared to females [152]. These findings provide evidence as to how genetic and environmental factor interactions result in sex-specific phenotypes of ASD. Male preponderance has intrigued hypotheses on a female-protective effect in ASD. Studies examining quantitative autistic impairments in twins of female and male genders provided evidence supporting the existence of a female-protective mechanism such that female probands need greater familial etiological load to present ASD-like traits [153]. Future studies of large representative samples are required for the accurate assessment of a female-protective effect in ASD.

Epigenetics provides a link between environmental changes and alterations to the chromatin, by regulating gene expression. Despite the mounting evidence suggesting a role for environmental factors in the pathology of ASD, concrete evidence is still missing as to what environmental factors are highly linked with ASD risk and what pathways they impact. Hence, future studies should integrate the available information on the influence of environmental factors, implemented in large samples carefully controlled for factors impacting the outcomes. The additive and synergistic effects of genetic, environmental, and epigenetics on ASD risk await further investigations. The application of animal models allows for the precise and quantitative modeling of environmental exposures. The interplay between genetic and environment in ASD is an area of active research, and the causality of these factors is yet to be verified.

Conclusion

During the past decade, genetic studies have identified many genes in ASD individuals with rare and common variants, predicted to contribute to the disease risk. However, for a substantial portion of these variants, the causality is yet to be verified. Studies of quantitative **endophenotypes** (i.e., biological traits indexing genetic susceptibility) are required in order to assess the contribution of the risk alleles to the pathogenicity of ASD. The application of RNA-sequencing technologies to determine expression variability of ASD variants is, therefore, an essential aspect of the future studies.

The variable expressivity and penetrance of rare variants observed in ASD subjects may be explained by differential contribution of common variants as well as epigenetic regulation, in addition to their interaction with the environmental factors. In the future, it will be essential to comprehensively assess genetic and epigenetic factors, and their combined effect in modulating ASD risk.

Today, we have access to vast genomics data from thousands of ASD individuals, which are rapidly growing as NGS technologies are becoming less expensive and more accessible. With the improvements in these technologies, we now can implement DNA methylation-sequencing data to generate information on the epigenetic alterations associated with ASD using large representative cohorts, providing a great resource for the identification of potential biomarkers that can be used for disease screening as well as monitoring responses to therapies. We are currently in the era of large genomics data, and the need for proper interpretation and functional characterization of this data is more essential than ever before. This challenge is exacerbated for the clinical interpretation of missense and noncoding variants, as well as TR expansions, given the difficulty in predicting their functional effects. Bioinformatics tools are currently being developed to efficiently and precisely analyze large amounts of data being generated every day for neurodevelopmental disorders, including ASD. In particular, the detection of structural variants and repeat expansions has been proved challenging due to their sequence complexity, and requires the development of accurate and reliable algorithms ensuring their precise detection. These tools will help us to uncover additional common genetic and molecular pathways enriched in ASD individuals, and thus, reliably manage and interpret genomics data.

Complementing bioinformatics and meta-data analyses, as well as integrative analyses of gene × environment influences with experimental approaches provides us with a deeper understanding of ASD etiology. The increasing use of cerebral organoids which mimic the structure and neural circuitry of the human brain provides an invaluable opportunity to explore the functional consequences of the genetic and

epigenetic variants in vivo. The application of cerebral organoids in neurodevelopmental disorders is particularly advantageous since rodent models of ASD have fundamental developmental and structural differences compared to humans.

In the future, the integration of neuroimaging studies with genome, epigenome, and transcriptome data, complemented with in vivo and in vitro characterization experiments will provide a comprehensive image of the ASD genetic structure. It will also be important to integrate what is known about ASD's (epi)genomic architecture with disorders often comorbid with ASD, especially epilepsy and schizophrenia, and to conduct studies to better understand this comorbidity and the single or polygenic effects of the implicated variants in ASD risk. The pleiotropic effect of genetic variants identified in ASD makes the phenotype–genotype correlation as well as functional characterization studies challenging. For example, in the case of ASD–epilepsy comorbidity, despite shared common biological pathways, it is still unknown whether there is a causal relationship between epilepsy and ASD, or whether the two disorders result from a common underlying pathology [154].

The following decades will witness outstanding improvements in our knowledge on neurodevelopmental disorders, including ASD. The global effort taking place today to delineate the genetic architecture of ASD will shed light on the molecular mechanisms underpinning these disorders and ultimately guide efficient diagnosis and treatment.

End-of-Chapter Summary

- Previous epidemiological studies of twins, siblings, and families provide evidence for a strong genetic heritability in ASD, where heritability estimates can range between 64 and 91%.
- ASD is comorbid with various genetic syndromes, which can be monogenic (such as Fragile X syndrome, Rett Syndrome, and Tuberous Sclerosis Complex) or complex in nature (including epilepsy and schizophrenia).
- Despite the 'common disease–common variant' hypothesis, common genetic risk variants confer a small effect individually but instead act additively to increase overall ASD risk.
- The advent of chromosomal microarray and NGS approaches have provided substantial evidence for the 'common disease–rare variant' hypothesis, where a number of rare but highly penetrant SNVs and CNVs have been implicated in ASD etiology.
- Other than SNVs and CNVs, indels, mtDNA defects, PZMs, TR expansions, and non-coding RNA variants have also been implicated in ASD etiology.
- To date, ~100 genes or loci are associated with ASD risk, where no single gene or locus accounts for more than 1% of all ASD cases.
- There is an enrichment of ASD risk genes in mechanisms regulating gene expression – especially in epigenetic mechanisms (such as chromatin remodeling, DNA methylation, and histone post-translational modifications), transcription factors, and alternative splicing.
- Variants in ASD risk genes also disrupt a number of synaptic and signaling pathways, including synaptic development, neuron circuitry, GABA-, and glutamate-mediated transmission.
- Several environmental factors are associated with ASD, including parental age, gut microbiota, sex-specific traits, and the maternal gestational environment (such as perinatal factors, maternal diet, and valproate exposure).
- Differential epigenetic regulation bridges the effects of environmental factors with gene transcription rate, through mechanisms such as chromatin remodeling.
- Despite the identification of multiple genetic, epigenetic, and environmental risk factors in ASD etiology, there is still much work to be done to complete our biological understanding of this common yet complex neurodevelopmental condition.

Multiple-Choice Questions

1. ASD is often comorbid with various genetic syndromes, such as Fragile X Syndrome (FXS). Which type of variation has been implicated as the molecular basis for FXS?
   (a) A single-nucleotide variant in the X-linked *MECP2*.
   (b) A duplication of the last intron in the X-linked *FMR1*.
   (c) **A CGG repeat expansion in the X-linked *FMR1*.**
   (d) The molecular basis for FXS is still unknown.
2. Which form of statistical analysis can be used to examine the combined effects of common SNPs?
   (a) A genetic burden test.
   (b) **A polygenic risk score (PRS).**
   (c) Pathway enrichment analysis.
   (d) None of the above.
3. Which of the following is NOT associated with ASD risk?
   (a) Prenatal maternal diet.
   (b) **Maternal negligence.**
   (c) Altered gut microbiota.
4. What is the relationship between parental age and the risk of ASD?
   (a) **There is a link between increased paternal age and ASD risk.**
   (b) There is a link between decreased paternal age and ASD risk.
   (c) There is no link between maternal age and ASD risk.
   (d) There is no link between paternal age and ASD risk.

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Preclinical Models of Autism Spectrum Disorder

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Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
ASD	Autism spectrum disorder
BDNF	Brain-derived neurotrophic factor
ChIP-Seq	Chromatin immunoprecipitation sequencing
CT	Computed tomography
dMRI	Diffusion magnetic resonance imaging
DNA-Seq	DNA sequencing
E/I	Excitatory/inhibitory
EEG	Electroencephalography
EP	Electrophysiology
ERP	Event-related potentials
fMRI	Functional magnetic resonance imaging
FMRP	Fragile X mental retardation protein
GABA	γ-aminobutyric acid
H&E	Haemotoxylin and eosin
IFG-1	Insulin growth factor
iPSC	Induced pluripotent stem cell
MECP2	Methyl CpG-binding protein 2
MIA	Maternal immune activation
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAA	N-acetyl-asparatate
PolyI:C	Polyinosinic:polycytidylic acid
RNA-Seq	RNA sequencing
VPA	Valproic acid

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Learning Objectives

1. Understand the limitations of clinical studies and how preclinical research is used to further our understanding of ASD.
2. Understand what makes a good preclinical model and what criteria researchers use to validate these models.
3. Identify the different animal models used to study ASD, as well as the different purposes they serve.
4. Learn how animal models are used to answer research questions, and what these questions are for ASD.
5. Identify and understand the principal methods used to assess animal models of ASD.
6. Understand why behavioural assessment is considered the gold standard, how it is performed and what limitations are associated with it.
7. Learn why treatment discovery is challenging and how these challenges can be overcome.
8. Identify the general patterns of neurobiological and neuroanatomical changes that were discovered using animal models of ASD.

Highlights

- Animal models play an important role in elucidating the pathophysiology of ASD.
- No single animal model can be used to represent ASD, but findings from multiple models can provide insight into the underlying mechanisms.

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- Genetic mouse models are used most commonly to study ASD.
- Behavioural assessment is the gold standard for model assessment but is associated with multiple limitations.
- Various assessment methods are available to probe the pathophysiology of preclinical models for ASD.
- Mouse models of ASD have been used to identify consistent changes in synaptic function, disruption of GABAergic pathways, imbalance in excitatory/inhibitory signalling and change in brain connectivity.
- Treatment development for ASD is challenging, but animal models have been used to identify common signalling pathways that can be targeted.
- Despite their limitations, animal models have played a significant role in ASD research and will continue to be useful in the future.

Introduction

Autism Spectrum Disorder (ASD) is characterized by a high degree of etiological and phenotypic heterogeneity. Investigating this complex heterogeneous space has been the aim of numerous clinical studies, which have proven instrumental in advancing our knowledge of the disorder [1]. One line of research has been to characterize ASD sub-populations on the basis of underlying genetic and epigenetic modifications [2]. Studies combining genetic screens with population statistics have identified over 900 genes implicated in ASD (SFARI database). These genes can then be used to define clusters of patients that share similar pathophysiological characteristics [3]. Complementing this approach, scientists have employed a range of neuroimaging modalities to explore the cellular processes underlying the neurodevelopmental changes observed in ASD [4, 5]. Yet another area of interest has been the assessment of potential risk factors and their impact on neurodevelopment. For example, longitudinal studies have examined the effect of prenatal stress, such as toxin exposure, on child developmental outcome [6]. Determining the significance of each prospective risk factor enables the formulation of improved prevention strategies and early intervention practices. Finally, clinical trials for candidate drugs provide useful feedback on the accuracy of our understanding of the underlying pathophysiological mechanisms [7].

Despite the wealth of understanding they have provided, clinical studies are faced with inherent limitations. They are constrained to using minimally invasive methods, such as behavioural assessment, neuroimaging and multi-omic screens, making it challenging to elucidate the underlying mechanisms of ASD. Histological assessment of human post-mortem specimens to obtain cytoarchitectural information is possible but limited due to the scarcity of samples. Moreover, the heterogeneity observed in ASD makes it difficult to disentangle the contribution or severity of every risk factor through clinical studies alone [8]. Clinical studies are additionally limited in sample size. Although ASD is highly heritable, the occurrence rate of each ASD-related genetic modification is rare. Thus, the number of patients constituting each prospective ASD sub-population is low, resulting in studies insufficiently powered to answer the research questions of interest. On the other hand, broader groups defined using less stringent classification criteria will naturally give rise to increased variability within each group. Finally, it is challenging to perform longitudinal studies using patient populations. These inherent limitations of clinical studies limit the types of research questions that can be investigated and the extent of the knowledge that can be obtained.

Faced with such limitations, scientific researchers have turned to a variety of preclinical models to answer deeper questions about the biology and pathophysiology of ASD [8, 9]. In particular, animal models have played a significant role in pushing the boundaries of what questions can be investigated. Contrary to human studies, preclinical studies allow for a large degree of experimental control. Gene-editing technologies have made it possible to have precisely controlled genetics, while standardized experimental procedures and animal husbandry allow for controlled experimental conditions. Thus, much of the intra-group variability and heterogeneity that plagues human studies is eliminated. This high level of control allows for each variable of interest to be tested separately while keeping all other factors constant [10]. As a result, most biological systems can be studied in terms of their constituent parts, which makes it possible for researchers to obtain insight into basic underlying mechanisms. In addition, a larger variety of experimental methods, both invasive and non-invasive in nature, can be used to assess a broader spectrum of experimental conditions than is feasible in humans [11–13]. Such information can lead to more informative comparisons of the various, often seemingly disparate, human cases, as well as to the identification of possible therapeutic targets. In addition, preclinical studies allow for custom sample sizes, providing the statistical power often absent from clinical studies of rare mutations where the number of participants is inherently limited [9].

In this chapter, we will discuss multiple aspects of preclinical studies. We begin by providing an overview of the types of animal models used in preclinical studies of ASD. We then discuss the methods most commonly used to assess these models. Finally, we present some of the principal findings that have resulted from studies using animal models.

Modelling ASD in Animals

The majority of preclinical studies for ASD are performed using animal models. Modelling autism in animals is a challenging task however due to the disorder's heterogeneity. Given the variability in clinical presentation and the absence of a unique and well-defined causative mechanism, there is no single animal model for ASD. In order to make the problem more tractable, researchers have developed a wide variety of animal models that each approximates some facet of the disorder, with the hope of illuminating a specific instance of its pathology. At present, there exist hundreds of animal models for the study of ASD (SFARI database), though not all models are equally robust in their capacity to recapitulate the characteristics of the disorder. For the purpose of evaluating the validity of preclinical models, scientists refer to three qualitative *validators*. These are face, construct and predictive validity [14]. Each of these validators describes a specific aspect of how a model approximates a human disorder. *Face validity* refers to the phenomenological aspects of a model that are similar to the disorder in humans [14, 15]. In other words, the face validity of a model refers to its ability to reproduce the human symptoms. In ASD, the absence of rigorous diagnostic biomarkers or endophenotypes means that face validity is evaluated largely based on behavioural phenotypes [16]. The *construct validity* of a model refers to how well the model recapitulates the etiological factors of the disease [14, 15]. For instance, a model with perfect construct validity would be developed using the exact etiological process that causes the disorder in humans. The final validator, *predictive validity*, is a measure of a model's ability to respond to established therapeutic pathways, e.g. pharmacological interventions [14, 15]. At present, there are no established pharmacological treatments for the core symptoms of ASD, and so the predictive validity of models for ASD remains unknown [16]. Thus, the validity of animal models for ASD is established using face and construct validity. However, the unknown and heterogeneous aetiology of the disorder means that it is unfeasible to develop a model that recapitulates all instances of autism. In spite of this, a number of animal models have been generated with perfect construct validity for particular genetic mutations known to cause ASD. Models with high construct validity can also conceivably be generated by exposing animals to well-established environmental risk factors. Such appropriately constructed models should also present with face validity. It is worth noting, however that not all models for ASD exhibit robust construct validity. Some animal models, such as the mouse BTBR T + tf/J inbred strain, were found to present naturally with traits related to ASD [11]. In cases such as these, the validity of the model is evaluated solely on the basis of face validity. Ultimately, no single animal model is able to capture all of the complexity of the human disorder. Taken together, however, the different models provide a way of approximating the disorder as a whole. Animal models of ASD can be broken down into two main classes: (1) Genetic models and (2) Environmental models [14].

Genetic Models

The majority of animal models for ASD fall under the category of genetic models. ASD is a highly heritable neurodevelopmental disorder, with concordance rates of 70–80% between monozygotic twins [15]. The genetic architecture of ASD is highly complex, however, and no single gene is responsible for all instances of the disorder. In spite of this, researchers have identified a clear genetic cause in approximately 20–25% of patients [17]. The underlying genetic modifications identified in ASD involve single-gene mutations, rare and de novo polymorphisms, common polymorphisms and copy-number variants [17]. In the case of single-gene mutations, genetic association and linkage studies in humans have uncovered a number of genes that exhibit high penetrance for ASD. Such genes code for proteins involved in a variety of functions, such as synaptic connection and cell-adhesion (NLGN1-4, NRXN1, *SHANK*3), signalling and development (*EN*2, *PTEN*), and neurotransmitters and receptors (*GABRB*3, *SERT*) [18]. Moreover, ASD is a common comorbidity for rare Mendelian neurodevelopmental disorders, including Fragile X Syndrome, Rett Syndrome, and Tuberous Sclerosis. Given the multiplicity of implicated genetic loci, scientists have created numerous genetic models based on the highly penetrant variants identified in human studies as well as the Mendelian disorders. This was made possible by advancements in genetic engineering biotechnologies such as Cre-Lox recombination and CRISPR-Cas9. Preclinical studies using such models have been dedicated to determining the function of ASD-related genes, as well as their contribution to behavioural phenotypes observed in the ASD population. For example, *Arid1b*, a gene with a strong association to autism, can be "knocked out", or "turned off", in a model organism and the downstream effects can be studied. Additional types of modifications can be used to obtain further insight into a gene's function, e.g. gene dosage effects. The most common genetic models have been generated using genetic modifications in *Fmr1*, *Tsc1*, *Tsc2*, *Pten*, *Nf1*, *Mecp2*, *Ube3a*, *Cacna1c*, *Nlgn3*, *Nlgn4*, *Nrxn1*, *Cntnap2* and *Shank3* [14, 19]. In some cases, e.g. *Ube3a*, the modifications induced directly mimic the mutations found in humans, while in others, the model is generated simply by knocking out a gene in which human mutations can be found, e.g. *Fmr1*.

Environmental Models

Although the high rates of heritability for ASD point towards an aetiology that is primarily genetic in nature, the absence of perfect concordance between monozygotic twins allows for the possibility of environmental contributions. As a result, researchers have examined the impact of a number of potential environmental risk factors. The majority of these factors play a role during the perinatal developmental period. At this stage in development, the central nervous system is highly susceptible to perturbation. Some of the potential risk factors identified in human populations include advanced parental age, maternal diabetes, maternal infection during pregnancy, medication use during pregnancy and exposure to toxins [6, 20]. It is important to keep in mind that although these factors have been found to have some association with ASD, the relationships vary in strength. Animal models can play an important role in clarifying these relationships, for instance by characterizing the strength of these associations and identifying the determining factors such as timing and duration of exposure. Although many potential risk factors have been identified, animal research has so far been focused on two types of environmental impacts. The first is the role of the maternal immune system, while the second is the influence of early exposure to medications and toxins [6, 14].

There is accumulating evidence that points towards the role of immune factors in the pathophysiology of autism. In particular, epidemiological studies have suggested a relationship between ASD and the maternal immune environment during gestation [21]. In order to elucidate the mechanisms underlying this association, scientists have created animal models to study the consequences of activating the maternal immune system during pregnancy. These models of *maternal immune activation* (MIA) are commonly generated by exposing pregnant animals to a number of agents that elicit an immune reaction. Agents used in the past have included a number of viruses and immunostimulants, including the influenza virus, cytomegalovirus, polyinosinic:polycytidylic acid (PolyI:C) and bacterial lipopolysaccharide [20].

In addition to elucidating the influence of the maternal immune system, animal models have been used to study the impact of prenatal exposure to medications and toxins. Among these, valproic acid (VPA), a common antiepileptic medication and mood stabilizer, has been studied most extensively [20]. As with the models generated to study the immune hypothesis of ASD, animal models for the study of VPA are generated by exposing pregnant mothers or embryos to the chemical. Other compounds that have been assessed using animal models include thalidomide, misoprostol and propionic acid [19]. Environmental models such as these are often used in conjunction with genetic modifications in order to characterize the interaction between genetic and environmental factors.

Animals Used to Model ASD

Over the years, ASD has been modelled using a variety of animal species, since different species provide different advantages depending on the aim of the study. Some important considerations include the genetics of the species, developmental trajectory, structural similarity to humans and spectrum of response to various stimuli [19]. The animals used most commonly in neuroscience research are *Caenorhabditis elegans*, *Drosophila*, zebrafish and the mouse, though studies have also been performed using rats and non-human primates [15]. However, the majority of preclinical models for ASD have been generated using the mouse as the model animal. Mice have become standard in biomedical research for a number of reasons. With regard to ASD, mice are social animals that exhibit clear signs of social interactions, allowing scientists to examine behaviours that are reminiscent of the human symptoms [18]. Moreover, they are easily housed, maintained, observed and manipulated. Their prolific breeding tendencies and rapid life cycles allow for high-throughput studies to be performed. Although these advantages can be said of the rat as well, the mouse became the animal of choice for autism research due to the comparative ease with which scientists could engineer its genome [22]. The ability to knock out specific genes in mice has led to the creation of numerous genetic models for ASD, as described above. While the mouse continues to be the animal of choice when it comes to genetic engineering, new biotechnologies such as CRISPR-Cas9 are increasingly being used to modify the genomes of a variety of animals, including rats and primates [23]. Such advances in genetic engineering will allow researchers to create genetic models using animals with more sophisticated behavioural repertoires. Even with increasingly powerful biotechnologies, however, specific genetic or environmental models will likely continue to be used to examine isolated etiological factors and disease constructs. Although each model provides valuable information about specific instances of ASD pathophysiology, a true representation of the human disorder must rely on the aggregation of evidence from numerous studies.

Methods for Assessing Animal Models

Presently, ASD is diagnosed exclusively on the basis of specific behavioural criteria. However, the disorder is associated with a range of underlying functional and neuroanatomical changes [8, 24, 25]. Naturally, these behavioural and biological changes also emerge in animal models of ASD. There exist many methods for assessing the various dimensions of pathology. Broadly, they can be categorized in terms of behavioural, molecular-anatomical and anatomical-functional methods.

Behavioural Assessments

Given the clinical definition of ASD as a behavioural disorder [24], it is imperative for researchers to be able to identify and assess analogous behaviours in animal models. This is accomplished using well-established *behavioural assays*, which are used to characterize key dimensions of animal behaviour [26], such as sociability, anxiety, cognition, reward, learning and memory [16]. Given that rodents are the animals most commonly employed to generate models of ASD, researchers have developed many assays to evaluate their behaviour. A detailed list of these assays is presented in Table 19.1.

Despite their inherent limitations, behavioural assays are the primary tool used to assess models of ASD. This is largely due to the absence of established biomarkers or endophenotypes for the disorder. Behaviours are often employed as a primary diagnostic measure because they can be assessed using fast and relatively low-cost tests. Researchers working with animal models will often employ assays meant to target behaviours recapitulating the core symptoms of the disorder. For example, for animal models related to autism, three tasks are usually employed that aim to measure animal sociability, communication, and restricted and repetitive behaviours [18, 26]. While animal behaviour can be a useful means of assessing model validity or examining treatment outcome, it is crucial to keep in mind that these behaviours are only a loose analogue of the complex behavioural patterns seen in the human disorder. This is especially true for rodents [18].

As described in the DSM-V [24], ASD is diagnosed on the basis of persistent deficits in social communication and interaction, as well as a pattern of restricted and repetitive behaviours and interests. These dimensions of behaviour are recapitulated in mice using specific assays. The *three-chambered task* is a test of sociability, used to assess reciprocal interaction and response to social queues [27]. Social communication is assessed in mice using *olfactory communication* and *ultrasonic vocalizations* [27, 28]. However, there is no accepted correspondence between these forms of communication and human vocal and visual communication. Finally, restricted and repetitive behaviours are assessed using assays that test for spontaneous motor stereotypies, repetitive behaviours, as well as assays examining more complex reversal learning paradigms [18]. Additionally, there are numerous assays that characterize other dimensions of behaviour, such as anxiety, cognitive function, reward, learning and memory. ASD patients often present with deficits along these dimensions in addition to the core symptoms, which is manifested in the observed clinical heterogeneity. Thus, assessing animal models along multiple dimensions of behaviour not only allows for better pheno-

Table 19.1 Phenotypes related to ASD in clinical populations and the analogous phenotypes in mouse models. Numerous rodent behavioural assays can be employed to characterize pathological behavioural dimensions [26]. Table adapted from "Annual Review of Animal Biosciences", chapter "Behavioral Phenotyping Assays for Genetic Mouse Models of Neurodevelopmental, Neurodegenerative and Psychiatric Disorders"

Human phenotype	Rodent phenotype	Rodent behavioural tasks
Unusual reciprocal social interactions	Sociability deficits	Three-chambered social approach task; Reciprocal social interaction; Male–female social interaction; Juvenile play (rats)
Stereotype, repetitive behaviours with restricted interests	Motor stereotypies and repetitive behaviours	Stereotyped movement (jumping, circling, backflipping); Repetitive activities (self-grooming, digging, marble burying)
Communication deficits (Speech–Language impairments)	Impaired communication	Olfactory discrimination; Response to social cues (ultrasonic vocalizations)
Anxiety	Anxiety	Open field; O-maze; Elevated plus maze
Motor impairments	Motor dysfunction	Open field test; Rotarod test; Balance beam test; Grip strength; Normality of gait test (footprint analysis)
Sensory impairments	Sensory tests	Hot plate; Ophthalmoscope; Acoustic startle; Pre-pulse inhibition
Learning deficits	Learning and memory deficits	Morris Water Maze; T-maze Spontaneous Alternation Test; Barnes Maze Test; Novel Object Recognition (NOR); Object Location Memory Task; Y-maze Spontaneous Alternation Test; Fear Conditioning (contextual, cued); Operant schedule-induced responding; Touch screen visual discrimination and reversal learning; Intra- and extra-dimensional set shift; 5-choice serial reaction time

typic characterization, but also provides a way to characterize the variability observed in the behavioural measures [16]. Fig. 19.1. depicts some of the common behavioural assays used.

Over the course of an experiment, behavioural tasks are generally performed from least stressful to most stressful, and the time between successive tasks is adapted to account for potential carryover effects [26]. Although behavioural characterization is an important aspect of working with animal models for ASD, it is important to keep in mind that animal models are limited in their ability to recapitulate the complex behavioural phenotypes seen among human patients with ASD [18].

Molecular-Anatomical Methods

One of the primary endeavours of autism preclinical research is to investigate the molecular basis of the disorder. To this end, one line of inquiry involves the use of "omic" biotech-

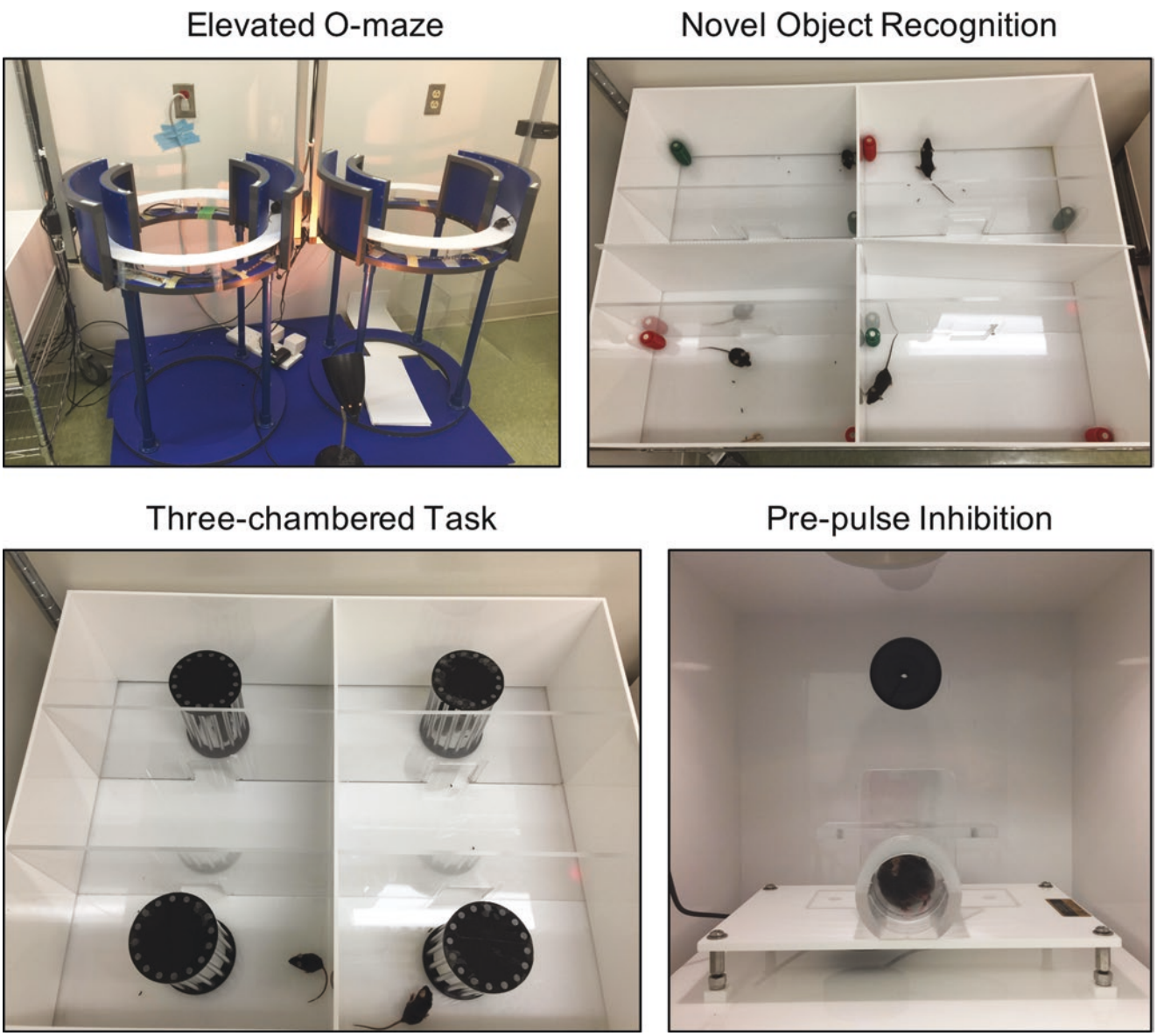

Fig. 19.1 A selection of behavioural assays commonly used to assess mouse models of ASD. Elevated O-maze relies on a mouse's fear of open spaces and heights to measure anxiety. Novel object recognition is used to assess learning memory. The three-chambered task is a canonical assessment for sociability. Pre-pulse inhibition measures adaption to sensory stimuli. Photographs by Tiffany Chien, Mouse Imaging Centre

nologies to characterize the genomic, transcriptomic and proteomic architecture of ASD. This is accomplished either by assessing changes in the various omic sequences using techniques such as DNA sequencing (DNA-Seq), RNA sequencing (RNA-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq), or by assessing levels of gene expression. The latter can be accomplished either directly by counting the number of cells that express a gene of interest, e.g. the reporter gene method [29], or indirectly by looking at the products of transcription and translation, e.g. northern and western blot analyses for RNA and protein levels, respectively [30, 31].

Another way to examine the molecular pathology of ASD is to probe the underlying cellular architecture by staining dissected, i.e. ex vivo, tissue. Staining is employed either for the purposes of *histology*, which is the study of the cellular structure [32], or for *immunohistochemistry*, which refers to the targeted visualization of specific molecules in cells [33]. In histology, the most common staining methods for neuronal tissue are H&E (Haemotoxylin and Eosin) and Nissl [34]. H&E staining is the principal method used and facilitates cell visualization by staining the cytoplasm in red and the nucleus in blue. The staining properties of cells are different under normal and pathological states. These properties also vary according to cell type. For instance, axons and dendrites cannot be visualized unless they are in pathological conditions that induce inflammation [35]. On the other hand, Nissl staining involves the staining of Nissl bodies, which are found on the rough endoplasmic reticulum of the cell. These are usually stained as cresyl violet [34]. Upon staining, cell bodies on the soma and dendrites can be visualized, but not those on axons, making this a preferred method for assessing neuronal density [36]. In contrast to histology, immunohistochemistry utilizes antibodies to tag and visualize proteins of interest. Briefly, each protein target expresses molecules called *epitopes* which are targeted by the highly specific *primary antibody*. The *secondary antibody* coupled to a reporter molecule binds to the primary antibody. It is the reporter molecule that reacts with the chemical substrate introduced, producing a colour precipitate, thus enabling visualization of the protein target. A large variety of antibodies, chemical substrates and reporter molecules are employed depending on the properties of the targeted protein molecule [33].

Finally, microscopy is used as a method to visualize tissue microstructure, primarily ex vivo. The tissue is visualized following excitation with either photon or electron beams (two-photon and electron microscopy, respectively). It can be used to visualize stained tissue, following the immunohistochemistry procedure, or unstained dissected tissue. The resolution and depth vary depending on the type of particle beam used [37]. Photon microscopy can also be performed in vivo, either with an intact skull or after exposing the brain section of interest [38, 39].

Functional-Anatomical Methods

The rapid advancement of medical imaging in recent decades has facilitated the use of neuroimaging to answer questions in neuroscience. In particular, neuroimaging has played an important role in elucidating the structural and functional characteristics of various psychopathologies, including ASD [11]. Modern neuroscientists have at their disposal a variety of imaging modalities to probe research questions of interest. In the context of ASD, these modalities can either be classified as *manipulation techniques*, such as optogenetics, or *signal readout techniques*, such as electrophysiology (EP) and magnetic resonance imaging (MRI) [4].

Manipulation techniques are modalities in which a change in neuronal function is induced in order to observe the associated neuronal response. The most common techniques are optogenetics, chemogenetics and pharmacological control.

Optogenetics is one of the more recent methods used for functional assessment. It is based on a form of neuronal control where the action of a cluster of neuronal cells is invoked using light of a certain wavelength, and the outcome, in the form of light of a different wavelength, is observed in real time. This is achieved by using genetic engineering to introduce light-absorbing and light-emitting, wavelength-specific proteins into the cells [40, 41]. As a result, it is possible to induce activity in a specific group cells and also monitor the response of a different group [42]. Most applications of this method leverage this ability to control neuronal cell activation/inhibition in order to interrogate neuronal circuits, both in normal and disease states, with respect to the network architecture and the signalling pathways, as well as explore new more tightly controlled interventions [41].

Chemogenetics follows the same principles as optogenetics. The main difference is that chemically engineered molecules (receptors) and ligands (specific to those receptors) are introduced into the cells, rather than light-sensitive molecules. Thus, neuronal function can be manipulated and controlled by introducing these new types of molecular interactions into the cells, with no further activation required [43, 44].

Pharmacological control involves the use of pharmacological agents in order to control the function of genes or of gene products. For example, a common agent is Tamoxifen, which is used to modify gene expression, by controlling the action of the gene editing protein, Cre. When Tamoxifen is

absent, the gene is "on", whereas when Tamoxifen is present, Cre is activated and the gene is removed (spliced) [45]. Targeted function is achieved by flanking the gene of interest by the loxP sites that are needed for Cre binding and function [46]. In a similar fashion, various other pharmacological agents are used. The goal of these manipulations is to interrogate gene function more closely but also to assess various substances for their therapeutic potential.

In contrast to manipulation techniques, signal readout techniques are imaging modalities by which neuroanatomy or neuronal function are assessed. The most common techniques are EP and MRI.

EP has traditionally been employed to assess brain function by interrogating the spatiotemporal patterns and rates of neuronal firing. Such methods provide unparalleled temporal resolution. EP can be performed on live animals or on dissected tissue that is artificially kept "alive". With this method, intra- or extra-cranial bipolar electrodes are used to detect the electromagnetic potentials created by a group of neurons that fire collectively. The measurements of these potentials can be described as event-related potentials (ERP) if the response follows a specific stimulus, or electroencephalography (EEG) if examining resting state functionality. EEG measures the changes in the electric field that arises as a result of neuronal firing. When using more invasive techniques, such as intracranial electrodes or dissected "live" tissue, greater resolution can be achieved by using bipolar microelectrodes that are placed in direct contact with single exposed neurons. This method allows for interrogation of a specific cell's electromagnetic activity, instead of the aggregate activity from multiple neighbouring cells. The neuronal firing patterns and rates measured provide information on synaptic signalling and plasticity, neuronal function and synchronization. In this way, numerous ASD-associated disruptions can be detected, such as the commonly observed excitatory-inhibitory (E/I) cell firing ratio imbalance. These measures can also act as surrogates for assessing other brain functionalities, such as connectivity and lateralization, often altered in ASD [12, 47–49].

MRI is not currently a gold standard for the assessment of animal models, but this modality has been gaining ground in recent years for rodent brain imaging. This is due primarily to the impressive spatial resolution with which the images can be acquired. Additionally, MRI facilitates the delineation of structures in the brain by means of superior contrast between distinct classes of soft tissue [50]. Image contrast can be modified in a number of ways, for instance by manipulating the imaging parameters and acquisition sequences, as well as by making use of contrast agents such as gadolinium and manganese [51]. Thus, a variety of information can be derived for different regions of the brain [52]. More recent advances in MRI methodology have been used to derive neurobiological information on a microstructural level, complimenting other ex vivo, invasive techniques like histology [50]. MRI is useful even in ex vivo studies, as it can be used for non-destructive examination of the fixed specimens [51, 52]. The most common MRI protocols used in neuroimaging are MR spectroscopy (MRS), diffusion MRI (dMRI), 3D volumetric MRI and functional MRI (fMRI) [11].

MRS is a method that allows for the quantification of neurochemicals with small molecular weight, given a sufficiently high field (spectral resolution) [53]. MRS can detect metabolites at concentrations of up to 10,000 times smaller than that of proton nuclei in fat and water molecules, which are detected with conventional MRI [54]. Common compounds in the brain are N-acetyl-aspartate (NAA), choline and creatine. MRS is also used to detect neurotransmitters and receptors, such as glutamate and γ-aminobutyric acid (GABA), which are integral parts of the central nervous system. The metabolite concentrations depend on the underlying biological conditions and can change as these conditions change, such as during development [55]. For example, glutamate and NAA/Creatine levels are markers of neuronal maturity and, thus, are used to assess brain development; decreased levels of glutamate, NAA/Creatine and NAA have been reported in immature neurons [56, 57]. Decreases in these metabolites have additionally been reported in various neurodegenerative disorders [55].

3D volumetric MRI, commonly obtained using T_1- or T_2-weighted sequences, provides information on gross volumetric changes for the whole brain or for individual brain regions. Image processing methods such as deformation-based and voxel-based morphometry are used to compute volumetric measures from T_1- or T_2-weighted MRI scans [50, 58]. An example of neuroanatomical changes obtained using 3D volumetric MRI and deformation-based morphometry is presented in Fig. 19.2. Here the colour maps indicate changes in brain volume for three mouse models of ASD, when compared to their respective wild-type controls.

dMRI complements conventional T_1- or T_2-weighted anatomical MRI by providing information about tissue structure at a sub-voxel level [59]. Specifically, this method relies on the process of water diffusion as a means of contrast, which is sensitive to the organization of tissue microstructures. The resulting signal can be used to infer about the existence and orientation of "ordered structures" within a voxel [59, 60]. The contrast obtained in this way is sufficient to

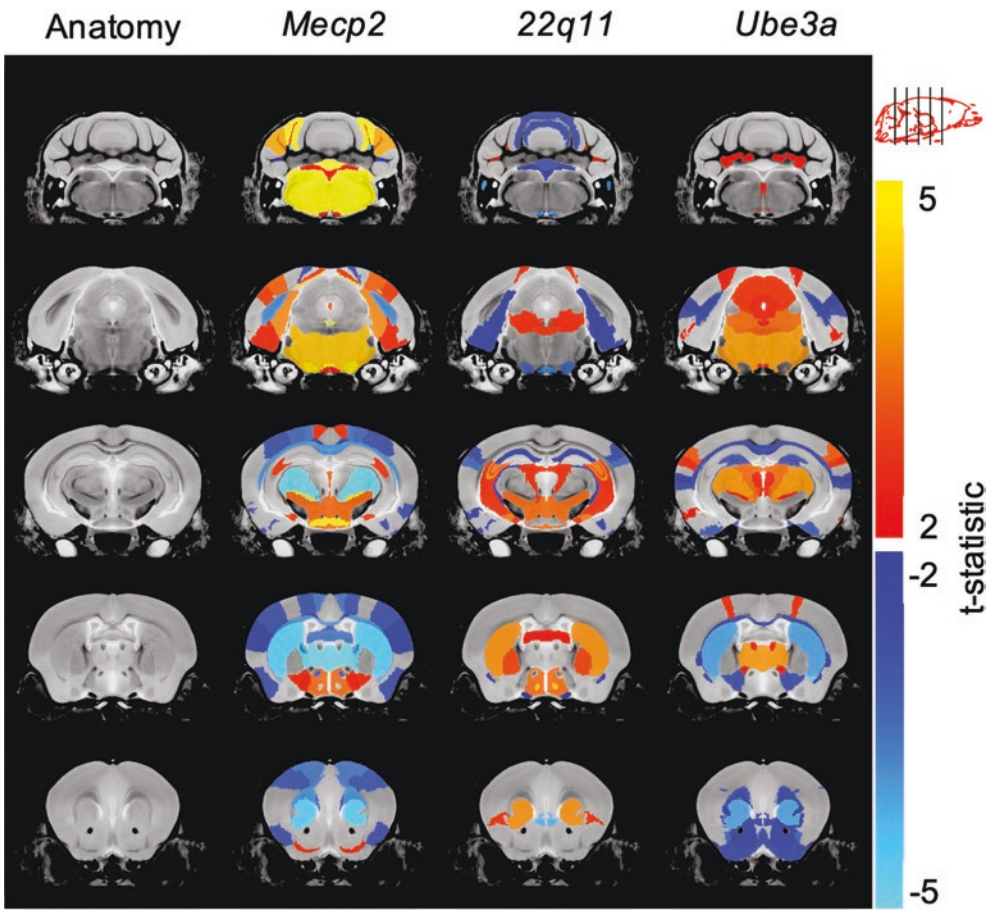

Fig. 19.2 Neuroanatomical changes associated with three mouse models of ASD. Differences in brain volume were evaluated by running independent linear regressions for every structure in an atlas. The regressor of interest was built to indicate membership to either the wild-type or mutant group. Thus, the t-statistic describes the effect size in a given region. Thresholds were selected to denote a minimum uncorrected p-value of 0.05, though massively univariate analyses of this type typically require correction for multiple comparisons. These changes highlight some of the heterogeneity associated with ASD. Similar regions exhibit morphological changes in the three models, albeit in opposite directions. Additionally, the *Mecp2* model is associated with more deficits in the cortex compared to the other two

distinguish internal structures within major brain compartments [61] (Fig. 19.3). For example, in early development, dMRI can be used to delineate the developing grey and white matter structures [60].

fMRI is an MRI protocol that is used to probe neuronal activity in real time There are three types of fMRI: (1) Resting state, which measures spontaneous neuronal activity without any external stimulus; (2) Stimulus evoked, which measures the change in activity following a specific external stimulus; (3) Pharmacological, which measures the change in activity following the administration of a pharmacological agent [62, 63]. In most cases, the signal measured in fMRI arises from the change in the ratio between oxygenated and deoxygenated blood. This ratio is affected by neuronal function. Specifically, neuronal activation leads to a local increase in oxygenated blood, thus, increasing the ratio. As a result, a larger signal will be detected in the associated brain region [63]. fMRI is performed using both awake and anaesthetized rodents. There is an ongoing debate regarding which state is more reflective of the human resting state [64].

Despite its versatility and advantages, MRI is not yet a staple imaging method for the assessment of rodent models due to associated technical limitations. For instance, it is technically challenging to translate the various methods used when imaging humans to the smaller scale needed to image rodent brains [65]. Another important limiting factor is the cost associated with the purchase and maintenance of an MRI system. When performing preclinical research, scientists often employ many of these assessment metrics, since they can be used to explore complementary aspects of pathology. Obtaining a variety of information also allows for correlations of the various metrics, potentially highlighting additional aspects of the complex system underlying the ASD phenotype.

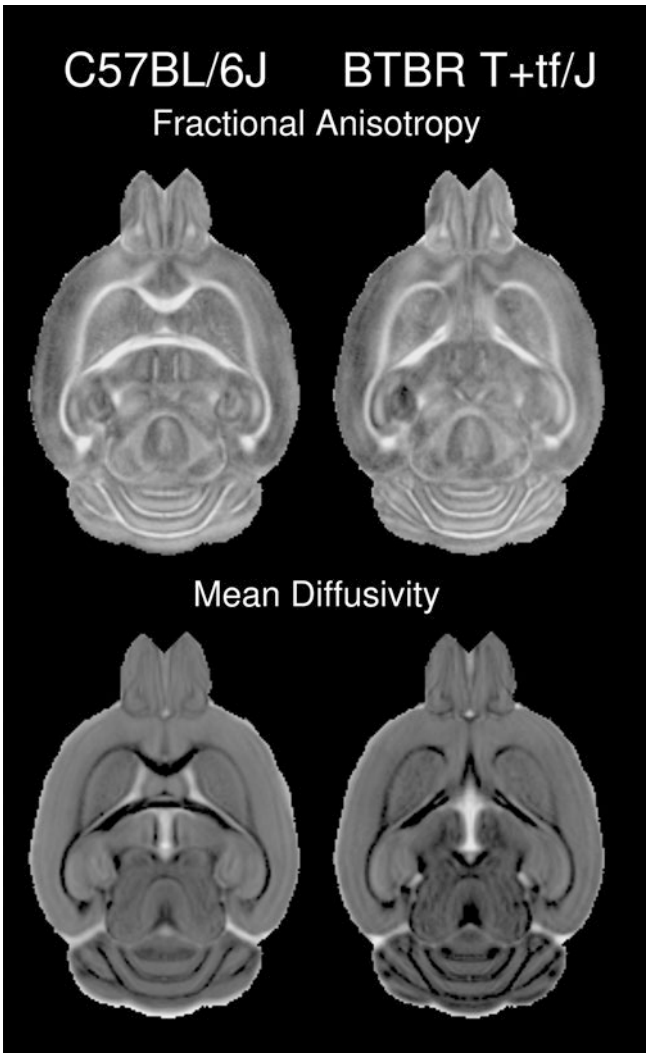

Fig. 19.3 Diffusion tensor imaging, a specific form of dMRI, is highly sensitive to microstructural organization in the brain. Fractional anisotropy and mean diffusivity are two types of contrast related to water diffusion that can be derived from this imaging protocol. The former is sensitive to the amount of directionality in the diffusion of water and, thus, provides strong signal for highly organized white matter structures. In contrast, mean diffusivity is a measure of the mean isotropic diffusion of water. Both contrasts clearly display the absence of the corpus callosum in the BTBR T + tf/J inbred strain

Findings from Preclinical Studies

Preclinical research performed using animal models, specifically mouse models, has resulted in a wealth of knowledge about the biological basis of ASD. Perhaps unsurprisingly given the heterogeneity of the disorder, this research has uncovered a diverse and complex landscape of pathology involving many different molecular, cellular and anatomical systems. So far, these findings have been discovered primarily from studies using genetic models of ASD, though environmental models are increasingly common [66, 67]. Each of these models is associated with characteristic disruptions in key systems, which in turn lead to specific hypotheses of dysfunction and potential therapeutic targets. Together, these preclinical models provide insight into the multiple mechanisms underlying ASD in the human population and can lead the way towards the development of novel therapeutic options.

Neurobiology and Neuroanatomy

Genetically modified mice have been instrumental in understanding the neurobiological changes associated with the disruption of specific genes involved in ASD. This is especially true of models recapitulating highly comorbid Mendelian neurodevelopmental disorders, such as Fragile X Syndrome. Most genetic models can be classified into four groups based on the functional changes induced: (1) Neuronal ion channels and receptors, (2) Synapse-related cytoskeleton and scaffolding proteins, (3) Epigenetic and transcriptional regulators and (4) Post-translational protein modifiers and regulators. Within each group, some models have been studied more than others, thus, providing a set of characteristic neurobiological changes for the group. An excellent review of the changes associated with different genetic mouse models can be found in Table 1 of Kim et al., 2016 [68]. Despite the evident heterogeneity, some common patterns can be discerned. A change in synaptic function is seen across all models. However, the direction and magnitude of the change vary depending on which gene is modified [66, 67]. The signalling pathway that is most commonly dysregulated in models of ASD is the GABAergic pathway. This occurs either directly, e.g. by GABA depletion, or indirectly, e.g. as a result of changes in other neurotransmitter levels, such as glutamate, which alter the outcome of the pathway's normal function [69]. Other pathways commonly affected are mGluR5, BDNF/TrkB and mTOR [7, 70–74]. The dysregulation of these neurotransmitter pathways leads to the commonly observed imbalance in excitatory/inhibitory (E/I) signalling, with various accounts suggesting a bias in favour of excitation [7, 69]. This E/I imbalance has been linked to the impairment of cortical function, sensory processing and integration, through various studies using mouse models [75].

Additionally, in many models, there is an observed change in short- and long-range brain connectivity, primarily in the cortex and the cerebellum. The direction of such changes varies with age and brain region [8, 76]. Other common changes include altered neuronal spine formation and Purkinje cell deficit, as well as elevated levels of platelet serotonin (5-HT) [8, 77]. Finally, immune dysregulation is also often observed in the brain and cerebrospinal fluid [8, 21].

Researchers have additionally focused on characterizing the neurobiology of ASD on the mesoscopic scale, by studying the morphological and volumetric changes in the brains of genetic animal models. Studies have reported neuroanatomical changes in mouse models of ASD, specifically in those models displaying ASD-like behavioural phenotypes. However, as seen in Fig. 19.2, the direction of these changes has been found to vary across models and brain regions [13, 78, 79]. In 2015, Ellegood et al. attempted to make sense of these anatomical changes by identifying common patterns between the various models [78]. Using 26 prominent genetic mouse models of ASD, they reported a clustering pattern based on voxel-wise morphological volumetric changes in 62 brain regions. These changes were captured using MRI and deformation-based morphometry techniques [9, 50, 80]. Three main clusters were identified that differed both in the direction of the volumetric changes and the location of the anatomical changes, reflecting the heterogeneity seen in the human population. These results are summarized in Fig. 19.4. The first cluster is characterized by increases in the cortex and decreases in cerebellum. This is in opposition to the third cluster, which exhibits the opposite pattern of deformation. Finally, the second cluster is characterized by decreases in many white matter structures as well as increases in subcortical areas [78]. It is noteworthy that the cerebellum was once again identified as highly implicated in ASD. Despite the plethora of findings on various scales described above, for most of these neurobiological changes the underlying genetic basis and circuitry is unknown [66].

Neurodevelopmental changes have also been observed in studies using environmental models of ASD, such as MIA or toxin exposure. These changes normally arise directly from the imposed stress or from changes in the immune system. The observed changes are along the same lines as those mentioned above for genetic models [6, 21]. For all types of models, the observed changes have also been observed in post-mortem studies of ASD patients [67, 68, 76].

Treatment Discovery

Mouse models play a significant role in discovering novel forms of treatment for ASD. This is especially true for interventions that target the underlying biology, such as pharmacological approaches or gene therapies. The process of treatment discovery is complicated, however. It would be ideal for clinical researchers to discover a single treatment that will benefit all patients with ASD, but the heterogeneity of the disorder makes this scenario highly unlikely. On the other hand, we might imagine a scenario in which a tailored treatment is developed for every genetic and environmental cause of ASD. Given the large number of etiological factors that play a role in autism, this is not likely to be feasible either. The most hopeful scenario is one in which scientists are able to classify patients into sub-populations that are defined on the basis of common mechanistic processes, which might arise from diverse genetic causes. This is commonly framed in terms of a few distinct molecular and circuit pathways, which could then be treated using a limited number of interventions [81]. As discussed previously, mouse models are crucial for understanding the neurobiological changes associated with specific genetic and environmental disruptions. Using inbred mouse strains with low genetic variability, it is possible to examine the biological changes associated with specific ASD-related genes, including disruptions in molecular and signalling pathways. Once these changes are well characterized for a given set of genes, scientists can develop mechanistically informed interventions to normalize the neurobiological and behavioural phenotypes. This approach has been explored most extensively using mouse models for Mendelian neurodevelopmental disorders that are highly comorbid with ASD. Important examples are Fragile X Syndrome (*Fmr1*), Rett Syndrome (*Mecp2*) and Tuberous Sclerosis (*Tsc1* and *Tsc2*). Each of these models has given rise to different theories of dysfunction and potential therapies [82].

Fragile X Syndrome is caused by a silencing of the *FMR1* gene, which results in the loss of fragile X mental retardation protein (FMRP). This disruption can be replicated using model organisms, including the mouse, by knocking out the homologous gene, *Fmr1* [83]. FMRP acts as a brake to the mGluR5 receptor, a metabotropic glutamate receptor. When FMRP is absent, excessive signalling of mGluR5 results in increased protein synthesis in dendrites and a higher density of dendritic spines [84, 85]. It also impairs long-term potentiation in the cortex and amygdala, and facilitates long-term depression in the hippocampus [82]. Deficiencies in GABA-mediated synaptic neurotransmission have also been observed in *Fmr1*-knockout mice [82, 85, 86]. Together these findings have led to two main strategies for therapeutic intervention: (1) Treatment with mGluR5 antagonists to reduce excessive signalling and (2) Treatment with GABA-receptor agonists to enhance GABA-mediated transmission [85, 87]. Numerous studies have shown that treatment of *Fmr1*-knockout mice with mGluR5 negative modulators was effective in improving neurobiological and behavioural phenotypes including reversing dendritic spine abnormalities, reducing seizures and reducing repetitive behaviours [82, 87, 88].

Rett Syndrome occurs primarily due to mutations in the X-linked gene methyl CpG-binding protein 2 (*MECP2*) [89]. The associated protein, MECP2, is a highly conserved nuclear protein that is expressed primarily in the brain. MECP2 serves many functions but plays a central role in

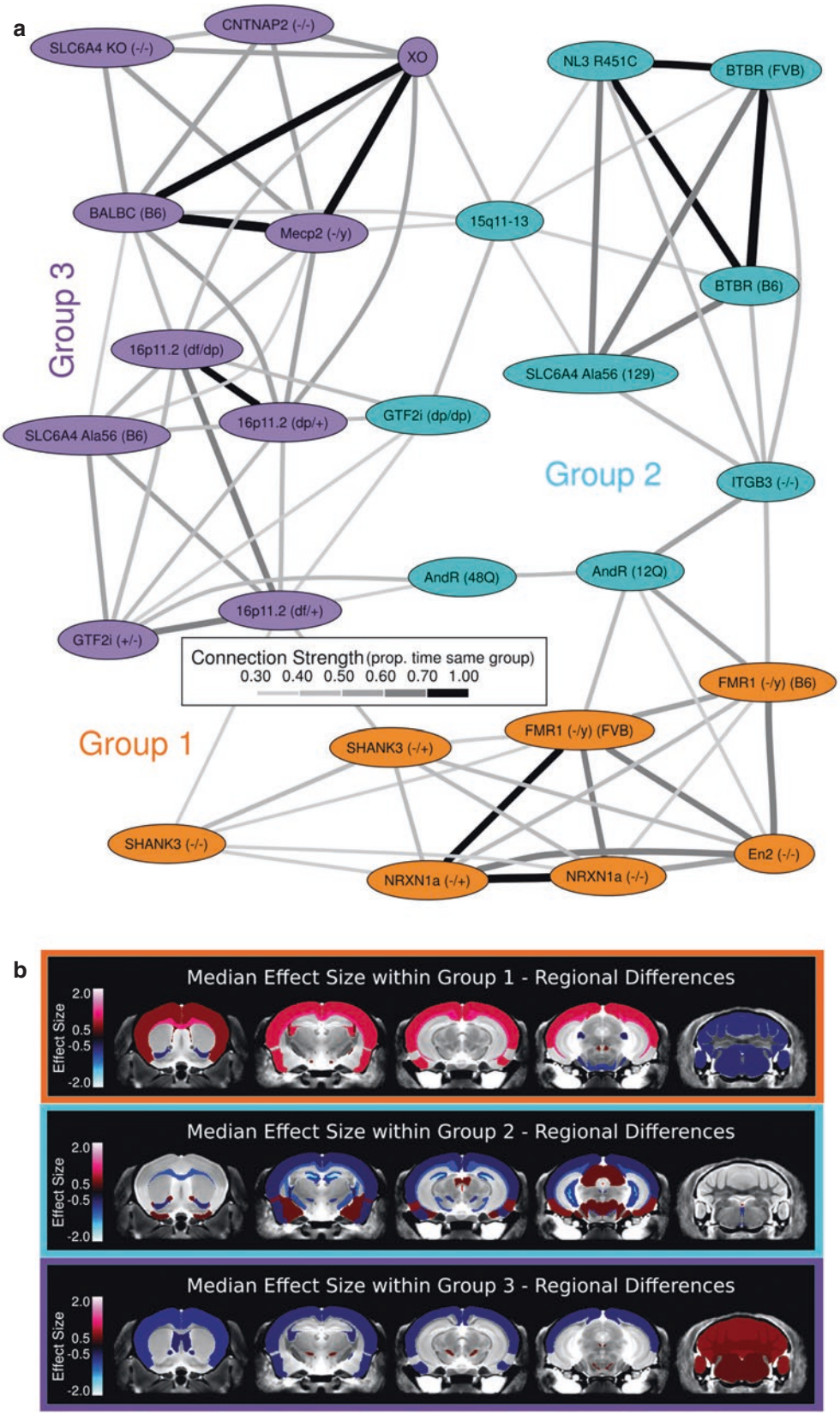

Fig. 19.4 Adapted with permission from Ellegood et al., 2015 [78]. (**a**) 26 mouse models of ASD were clustered together on the basis of neuroanatomical changes measured when comparing to littermate wild types. The clusters were generated using hierarchical clustering and assessed for robustness using bootstrap resampling. The connection strength measures how often models were clustered together in the bootstrapped samples. (**b**) Structure-wise differences in brain volume for each of the clusters, computed as the median of the effect sizes for all models in a given cluster

regulating gene expression through multiple mechanisms [88, 90]. Studies involving mice with a genetically engineered deficiency in *Mecp2* have identified deficits involving the structure and function of neuronal synapses [88, 91, 92]. Preclinical treatment strategies have focused either on targeting the direct loss-of-function mutation, or on targeting the downstream effects of *Mecp2* loss [88]. In particular, using conditional knockout models, it has been shown that reintroducing a functional copy of *Mecp2* later in development can reverse many morphological defects and improve behavioural phenotypes [93, 94]. Additional therapeutic approaches have focused on normalizing the levels of brain-derived neurotrophic factor (BDNF). The associated gene, *Bdnf*, is one of direct targets of MECP2 transcriptional regulation [88, 90]. Reduced levels of BDNF are thought to contribute to the pathophysiological mechanisms of Rett Syndrome [95]. Over-expression of BDNF in mice-lacking *Mecp2* has been shown to improve their phenotypes [96, 97]. However, this approach is limited by the low blood–brain permeability of BDNF. Alternative approaches have focused on using Insulin Growth Factor-1 (IFG-1), which activates a similar signalling cascade to BDNF and is able to pass through the blood–brain barrier [88].

Tuberous Sclerosis is a neurodevelopmental disorder caused by mutations in the *TSC1* or *TSC2* genes. The disorder is characterized by the presence of non-malignant tumours in the brain and other organs [98]. Mutations in *TSC1/2* lead to disinhibition of mTOR signalling, which can lead to cell body hypertrophy, low spine density and increased expression of AMPA receptors [82, 99]. Mouse models of Tuberous Sclerosis have been generated by knocking out *Tsc1* or *Tsc2* [87]. In either model, the deficits can be rescued by using mTOR inhibitors, such as rapamycin [82, 100].

The mouse models for these neurodevelopmental disorders have been studied most extensively for the purpose of discovering treatments that might be well suited to ASD. However, the same principles have been applied using other genetically engineered mouse models. Some important models include those with mutations in *Cntnap2*/*Caspr2*, *Nf1*, *Pten*, *Shank3* and *Ube3a* [87, 94]. In addition to pharmacological interventions, environmental and social enrichment strategies have been used to reverse abnormal phenotypes in mouse models of ASD. Environmental enrichment usually entails rearing animals in an environment that is more complex than standard housing cages. These enriched cages often include larger space, equipment to climb and explore, toys and running wheels. Enrichment can also be accomplished using novelty, by modifying the cages on a regular schedule. Social enrichment involves pairing mice with low sociability (e.g. BTBR T + tf/J), to mice with high sociability (e.g. C57BL/6 J) [94, 101]. In wild-type mice, environmental enrichment has been found to enhance learning and memory [102]. Studies have also reported that environmental and social enrichment have reduced behavioural and neurobiological phenotypes in *Fmr1*-knockout mice and *Mecp2*-knockout mice. These strategies improved exploratory behaviour while reducing anxiety and hyperactivity [103, 104]. They additionally improved abnormal dendritic branching in the *Fmr1*-knockout mice and normalized hippocampal BDNF protein levels in *Mecp2*-knockout mice [94, 101, 103, 104].

Conclusions

ASD is a complex neurodevelopmental disorder associated with diverse causes and clinical presentations. Despite its growing prevalence, it is not yet fully understood. While clinical studies are limited in their capacity to clarify the biological basis of the disorder, preclinical models, specifically animal models, have been instrumental in this endeavour. Animal models for ASD can be generated by imposing genetic or environmental perturbations on a variety of animal species, with the mouse being used most commonly [14]. These models have allowed scientists to probe the neurobiological deficits and behavioural phenotypes associated with ASD. In order to assess the changes resulting from specific etiological constructs, a suite of methodological tools is available. Behavioural assays are used extensively to assess the animal behaviours analogous to the symptoms of ASD [26]. To assess the neurobiology of these models, numerous biotechnologies and imaging modalities are used, including multi-omic sequencing, tissue staining, EP and MRI. Common neurobiological changes have been identified, including change in synaptic function, disruption of GABAergic pathways, imbalance in excitatory/inhibitory signalling and change in short- and long-range brain connectivity. Animal models also play a significant role in treatment development for ASD symptoms. In this case, the strategy has been to identify in these models prospective molecular targets that are highly comorbid for autism, with the hope that such interventions will generalize to the greater population of patients with ASD [81, 82]. This approach has produced pharmacological compounds that impair mGluR5 signalling, enhance GABAergic transmission, restore transcriptional regulation and inhibit the mTOR signalling pathway. However, despite greatly improving our insight into ASD and its underlying mechanisms, these animal models are not without limitations.

Given the complexity and heterogeneity of ASD, the disorder cannot be approximated using a single animal model. Although part of the autism spectrum is likely caused by polygenic factors, these are not yet well understood, nor modelled at all in the mouse. As a result, knowledge about the pathophysiology of autism must be obtained by the aggregate findings from models of *single-gene dysfunction*,

which serves as an approximation to the broader spectrum. However, even though these genes can be disrupted in a tightly controlled manner, the resulting phenotypes must always be interpreted within the context of the genetic background of the chosen inbred strain. Under the assumption that a given gene variant has a large effect and that interactions with the background are negligible, the resulting neurobiological deficits should be consistent across different inbred strains. However, phenotypic differences have been observed between models generated using the same disease construct on a different background strain. This is especially true for behavioural phenotypes, since behaviour is removed far enough from the initial model construct that small perturbations at multiple scales will be amplified to give rise to a large degree of variability. In addition, variations in the environment, e.g. lab protocols, can also give rise to differences in the observed phenotype. This inherent variability in phenotype, especially behaviour and susceptibility to environmental differences often result in inconsistent findings across different research groups, even when studies are performed using the same mouse model. Given that there are no established biomarkers for ASD, the face validity of mouse models must be established using behavioural assays. These assays are useful for measuring specific dimensions of mouse behaviour, but it is not obvious what dimensions of human behaviour they translate to and whether they truly recapitulate the clinical symptoms of ASD. As a result, scientists have no robust way of establishing strong face validity. This is problematic for studies that attempt to identify potential etiological factors or validate new ASD models on the basis of behavioural outcomes. The main reason is that the correspondence between the observed neurodevelopmental and behavioural changes, and specific neurodevelopmental or neuropsychiatric disorders is not clear.

To address these limitations, scientists are exploring novel avenues of research. Although preclinical research has been conducted using the mouse as the primary model animal, studies using rat models are increasingly common. As mentioned previously, this is mainly a result of advances in genetic engineering technologies, which have facilitated the creation of genetic rat models. Rats exhibit more complex social behaviours than mice and have a richer set of behavioural phenotypes. Testing for communication skills is expected to be more sensitive in rats than in mice; thus, rat models are expected to have stronger face validity than mouse models [16]. In addition to rats, researchers have proposed using non-human primates as a bridge between rodent models and human populations, as they are more closely related to humans, both in terms of genetics and in terms of neurobiology and neuroanatomy [105]. Not all advances in preclinical research are focused on animal models, however. Scientists are increasingly using induced pluripotent stem cells (iPSC) to model ASD. iPSC are somatic cells obtained from individual human patients that are then reprogrammed to become pluripotent stem cells. Thus, they can serve as more accurate cellular models under diverse conditions. They can also be used to examine in greater detail the cellular phenotypes associated with ASD. Importantly, iPSC can be combined with in-vitro EP to examine patterns of action potential firing, synaptic transmission and activity-dependent synaptic plasticity [106].

Both clinical and preclinical research studies have contributed significantly to our understanding of the complex disorder that is ASD. Although each of these avenues of research is faced with specific limitations, they provide complementary information that helps refine our knowledge of the disorder. As research into ASD moves forward with advancements in methodology and theory, studies using preclinical models will continue to serve an important role in addressing research questions that aren't feasible to tackle using clinical populations.

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Multiple Choice Questions

1. What criterion for animal model validity is associated with recapitulating the symptoms of the human disorder?
   (a) Predictive validity.
   (b) Face validity.
   (c) Construct validity.
   (d) None of the above.
2. Which of the following statements about rodent models of ASD is **false**?
   (a) Rodent models of ASD lack predictive validity.
   (b) No single model is representative of ASD.
   (c) No common phenotypes have been discovered between the different rodent models.
3. Of the following statements about methods used to assess rodent models of ASD, identify the **correct statement**.
   (a) Behavioural assessment is the gold standard for evaluating rodent models because rodent behaviours resemble human behaviours.
   (b) Computed Tomography (CT) is an established method used to probe gross neuroanatomical changes in rodent models of ASD.
   (c) Using electrophysiology (EP), functional information can only be obtained in vivo.
   (d) Magnetic Resonance Imaging (MRI) can be used to obtain information about chemical composition.
4. Common patterns of disruption have **not been found** in preclinical models based on:
   (a) Neuroanatomical changes.
   (b) Molecular and cellular changes.

(c) Synaptic and circuit-level changes.
(d) Behavioural phenotypes.

5. All candidate treatments for ASD discovered using preclinical models are:
   (a) Unique to specific models of ASD.
   (b) Behavioural in nature.
   (c) Based on common disrupted systems.
   (d) Also tested in humans.

Answers 1-C, 2-A and B, 3- A, 4-C, 5-A.

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Biomedical Interventions for Autism Spectrum Disorder

20

Janys Joy Lim and Evdokia Anagnostou

Learning Objectives

- Discuss target symptoms for biomedical interventions.
- Discuss evidence for currently used medications, indication for use, and side effects in the context of ASD.
- Briefly overview complementary and alternative biomedical intervention in ASD.
- Review translational efforts for the development of biomedical interventions for core symptoms.

Highlights

- ASD is a highly heterogeneous, lifelong neurodevelopmental disorder characterized by social communication deficits, and restricted and repetitive behaviors.
- Highly co-occurring symptoms include, but are not limited to, irritability, ADHD symptoms, anxiety and depression, seizures, sleep difficulties; they may be predicted by the underlying heterogeneous neurobiology of ASD, and may impact core symptoms.
- Available medications used in ASD mainly manage co-occurring symptoms. There are no medications available for core symptoms of ASD.
  - There are only two medications approved by the FDA for use in managing irritability and aggression in ASD, risperidone, and aripiprazole. However, they are associated with significant morbidity. Canadian guidelines for monitoring safety should be followed when prescribing such medications.
  - Randomized controlled trials in ASD also support medications for the treatment of ADHD symptoms (stimulants, atomoxetine, alpha agonists).
  - Common comorbidities such as anxiety, depression, seizures, and sleep difficulties should be managed similarly to neurotypical populations in the absence of specific ASD data.
- Complementary and alternative medical treatments are commonly used and in the absence of robust evidence, a risk-to-benefit assessment is important when counseling patients.
- New translational approaches are underway to utilize the exposing of genomic and systems neuroscience findings to develop targeted treatments in accordance with the principles of precision medicine.

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Introduction

Autism Spectrum Disorder (ASD) is a lifelong neurodevelopmental condition characterized by difficulties with social communication, as well as restricted and repetitive behaviors. The condition is highly heterogeneous with both genomic and environmental factors influencing its prevalence and phenomenology [1]. Heterogeneity is not

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only seen in underlying biology but also in presentation. In addition to core symptom domains, children, youth, and adults with ASD often present with other behavioral and physical differences that may cause dysfunction or impairment, such as ADHD symptoms, irritability/aggression, anxiety and mood dysregulation, as well as gastrointestinal distress, sleep disturbance, epilepsy, and immune differences. It is not clear yet whether such symptoms are truly comorbid or whether they are part of a large spectrum of neurodevelopmental differences that co-occur with social deficits and repetitive behaviors, co -present across neurodevelopmental conditions (NDDs), potentially arising from overlapping biologies, and are responsible for the heterogeneity seen in ASD and across NDDs. No medications have been shown to be effective for core symptom domains, although this is an active area of research [2]. This is not surprising given the limited understanding of the neurobiology of the condition. Still, the explosion of genomic and systems neuroscience findings suggest potential targets that will likely lead to the development of compounds targeting underlying neuropathology and, therefore, more likely to impact core symptom domains. For now, most medications available with robust evidence for use in those with ASD have emerged out of observations of phenotypic similarities in common symptoms in those with ASD and other conditions (e.g., attention/hyperactivity deficits, chronic irritability), and primarily include agents targeting dopamine and serotonin systems. In this chapter, we will review the evidence for the use of such medications for co-occurring symptoms and discuss active research aiming to target core biology of ASD to impact a variety of domains producing distress.

Where We Have Evidence for Effective Medications (Table 20.1)

Irritability

Irritability is a common concern often requiring psychotropic medication use. It is typically defined as outbursts of anger, frustration, and distress; [3] and may or may not be associated with aggression. In most medication trials, irritability/aggression has been considered together as an outcome [4]. Such symptoms are associated with poor quality of life, increased parental distress, and may compromise educational and home placements [3]. Assessment of irritability/aggression should include assessment of medical issues (e.g., pain, gastrointestinal distress, poor sleep, seizures), mental health (e.g., co-occurring anxiety, ADHD), sensory dysregulation, communication ability, and access to appropriate communication systems, as well as understanding of antecedents and consequences, typically assessed through functional behavioral analysis. Behavioral approaches are the cornerstone of treatment of challenging behaviors to assure necessary skill acquisition and appropriate environmental modifications. Therapeutic approaches should include treatment of co-occurring conditions and management of sensory dysregulation.

In the case of moderate to severe chronic irritability, FDA has approved two atypical antipsychotics, risperidone and aripiprazole. The use of these two medications is supported by two Cochrane reviews, as well as an updated comparative effectiveness review by the Agency for Health Care Research and quality (AHRQ) in 2017. Specifically, a Cochrane meta-analysis in 2007 [5] on risperidone showed improvements in

Table 20.1 Medications with RCT evidence for co-occurring symptoms in ASD

Target symptoms	Medication class	Medication	Level of evidence	Effect size	Common adverse effects
Attention deficits/ hyperactivity	*Stimulants*	Methylphenidate	≥2 RCTs	Medium	Initial insomnia, anorexia, irritability
	Non stimulants SNRI	Atomoxetine	≥2 RCTs	Medium	Anorexia/nausea, Irritability
	Non stimulants alpha agonists	Guanfacine	1 RCT	Large	Sedation, hypotension, midcycle insomnia
		Clonidine	*No RCTs* [a]		Sedation, hypotension
Irritability/ aggression	*Atypical antipsychotics*	Risperidone	≥2 RCT	Medium to large	Sedation, weight gain/metabolic dysfunction, hyperprolactinemia, movement disorders
		Aripiprazole	≥2 RCTs	Medium to large	As above
		N-acetyl-cysteine	≥2 RCT Small		Nausea, vomiting
Initial insomnia		Melatonin	≥2 RCTs	Large	

[a]included as extended release clonidine is FDA approved for treatment of ADHD in children 6–17 years

irritability, with gains also in secondary outcomes such as hyperactivity, social withdrawal, stereotypy, and inappropriate behaviors in children with ASD and severe irritability. Some improvement in self-injurious behaviors have been reported in adults, [6] although the evidence in children and youth is limited. However, the use of risperidone is known to be associated with metabolic syndrome, weight gain, gastrointestinal symptoms (e.g., constipation, diarrhea), somnolence, drowsiness, dry mouth, nasal congestion, orthostatic hypotension, changes in prolactin levels, and extrapyramidal symptoms. Rarely, this may include neuroleptic malignant syndrome. The effective dose range in this population is between 0.5–3 mg/day (e.g., [7]). In 2012, a second Cochrane collaboration confirmed a reduction of irritability, with secondary improvements in hyperactivity, stereotypy, and inappropriate speech, with aripiprazole [8]. Side effects in the use of aripiprazole include weight gain, risk of metabolic syndrome, sedation, drooling, orthostatic hypotension, and tremors. It is suggested that daily dosing starts at 2 mg with most common effective doses in the RCTs to be 5 and 7.5 mg/day and range up to 15 mg a day. In Canada, there are published guidelines for the monitoring of safety of atypical antipsychotics in children and youth. (camesaguideline.org, Table 20.2) In the case where atypical antipsychotics are associated with significant weight gain, but cannot be withdrawn, a randomized controlled trial supports the use of metformin as an adjunct medication to improve BMI [9]. Of note, the addition of parent training in behavioral modification may improve the response to antipsychotic medications [10].

A systematic review and meta-analysis [11] examined the evidence for anticonvulsants in children with ASD to manage agitation/irritability. It suggested inconsistent findings in the use of valproic acid, lamotrigine, and levetiracetam. However, the small sample sizes for the valproic studies and the large placebo effect in the lamotrigine trial limit interpretation of these studies.

Lastly, weak evidence exists to support the use of alpha agonists, especially in children with arousal dysregulation [3] and N-acetyl-cysteine [12] an anti-oxidant and a modulator of glutamatergic neurotransmission.

Table 20.2 CAMESA guidelines for monitoring atypical antipsychotics in children and youth (modified from camesaguideline.org, example for risperidone)

Parameter	Pre - Treatment Baseline	1 Month	2 Month	3 Month	6 Month	9 Month	12 Month
Physical Examination Maneuvers:							
Height (cm)							
Height percentile							
Weight (kg)							
Weight percentile							
BMI (kg/m^2)							
BMI percentile							
Waist Circumference (at level of umbilicus) (cm)							
Waist Circumference percentile							
Systolic Blood Pressure (mm Hg)							
Systolic Blood Pressure percentile							
Diastolic Blood Pressure (mm Hg)							
Diastolic Blood Pressure percentile							
Neurological Examination:							
Neurological Exam completed?							
Neurological Exam Normal or Abnormal?							
Laboratory Evaluations:							
Test							
Fasting Plasma Glucose							
Fasting Insulin							
Fasting Total Cholesterol							
Fasting LDL-C							
Fasting HDL-C							
Fasting Triglycerides							
AST							
ALT							
Prolactin							
Amylase							
Other (e.g. A1C, OGTT, etc.); ***Please List***							

Attention-Deficit Hyperactivity Disorder (ADHD) Symptoms

There is high co-occurrence of ADHD and ADHD-like symptoms (15–85%) in children and youth with ASD, including hyperactivity, impulsivity, and inattention [13]. Up to 50% of children diagnosed with ADHD also have ASD traits. Thus, a number of studies examined whether medications approved for ADHD are useful in managing ADHD-like symptoms in ASD [14]. Current evidence supports the use of psychostimulants, atomoxetine, and alpha agonists in the treatment of ADHD symptoms in this population.

Psychostimulant medications, particularly methylphenidate, are effective in treating hyperactivity and inattention symptoms in ASD. Medium to high doses are associated with most benefit. Children with co-occurring intellectual disability may still benefit but have a less favorable response to methylphenidate, and seem to be more sensitive to adverse effects. Side effects include cardiac arrhythmias, anorexia, insomnia, and with chronic use, irritability, and mood lability. Systematic reviews suggest higher rate of drop out in the methylphenidate group compared to placebo due to side effects [14]. Family history of cardiac disease or sudden death should trigger consideration of a baseline ECG and a consultation with a specialist prior to initiating treatment [15].

Atomoxetine is a norepinephrine reuptake inhibitor that has also been studied in children with ADHD-like symptoms and ASD. Synthesis of four randomized controlled trials documented small to medium effect size improvements in both hyperactivity and inattention symptoms [14]. Better efficacy is associated with higher doses within the approved range. Common side effects include anorexia/gastrointestinal distress, as well as headache, fatigue, and irritability.

Alpha-2 adrenergic agonists (e.g., guanfacine and clonidine) also show some efficacy when used for hyperactivity in children with ASD. One RCT supports the use of long-acting guanfacine in this population [16]. The most common side effects of this class of medications include drowsiness and fatigue, although midcycle insomnia was also reported in the RCT of long-acting guanfacine. In some children, however, this class of drugs may cause hypotension, bradycardia, and syncope. Children also should be weaned from these medications as abrupt discontinuance of intake may cause rebound hypertension. The Canadian ADHD Resource Alliance (CADDRA, https://www.caddra.ca/pdfs/Medication_Chart_English_CANADA.pdf) and Health Canada recommendations suggest the maximum dose of long acting guanfacine is 4 mg/day for children up to 12 years of age and 7 mg/day for those older.

Atypical antipsychotics are occasionally used in this population for this domain. Caution should be exercised as any evidence of efficacy comes from trials targeting irritability, using hyperactivity as a secondary outcome, and therefore, such data should not be used as evidence of efficacy of this class for ADHD symptoms in children and youth who would not be otherwise prescribed such medications [15].

Repetitive Behaviors

Repetitive behaviors are a heterogeneous domain, which includes ritualistic behaviors, restrictive interests, sensory differences, and stereotypy. A systematic review of serotonin reuptake inhibitors (SSRIs) suggested no benefit compared to placebo for this domain [17]. Use of these drugs are associated with adverse effects that include gastrointestinal distress, agitation, disinhibition, insomnia, and sexual dysfunction, therefore, not supporting a favorable risk-to-benefit ratio in this population for repetitive behaviors. It is important to know that the domain does not include co-occurring OCD, and in the absence of clinical trials for comorbid OCD, these drugs are being used as in neurotypical youth for this condition. Atypical antipsychotics, such as aripiprazole and risperidone, were shown to have some effect on the domain of stereotypy in clinical trials targeting irritability [5]. However, controversy remains about the risk-to-benefit ratio of using such medications for stereotypy, as behaviors in this domain are not always associated with distress and/or interfere with function. In fact, autistic adults have reported that such behaviors may be employed effectively for emotion and arousal regulation. A functional behavioral analysis is often helpful to understand such behaviors before treatment is attempted.

Anxiety and Depression

Anxiety is one of the most common co-occurring symptoms in ASD, with a prevalence that can range between 39% and 84% in this population [18]. The primary intervention in the management of anxiety still remains modified cognitive behavioral therapy, with various RCTs supporting its use in verbal children and youth with ASD. However, the evidence remains limited for youth with poor language skills or poor introspection. There are currently no published RCTs of medications for the treatment of anxiety in this population, although several are in progress at this time. The Autism treatment Network published consensus practice parameters for the assessment and treatment of anxiety in this population in 2016 [18] based on available data and expert opinion. SSRIs, as well as treatment of arousal dysregulation (alpha agonists, beta blockers) and sleep disturbance associated with anxiety have been recommended, acknowledging the evidence gap.

Depression is also highly comorbid with a diagnosis of ASD. Prevalence estimates vary due to autistic symptoms scoring on depression instruments, but also diagnostic overshadowing attributing depression to autistic symptoms. High index of suspicion is required. Greater chronological age, and higher IQ, as well as greater rates of seizure and gastrointestinal problems are associated with the diagnosis in children and youth [19].

Evidence for treatment of depression in ASD is scarce with some emerging evidence for psychotherapeutic approaches, but very little evidence about what pharmacological interventions may be effective in this population [19]. As such, most clinicians employ treatment algorithms from the neurotypical population.

Seizures

Epilepsy and ASD are highly comorbid, with a pooled prevalence of epilepsy between 8% and 24% depending on the presence and severity of intellectual disability. The single most important risk factor for epilepsy is that this population is impaired cognitive ability [20]. Available data to expert consensus suggest that epilepsy and ASD share pathophysiologic mechanisms, from genomic architecture to immune mechanisms. Treatment of the seizures in this population is not different from that of neurotypical children with epilepsy. However, it is important to note that some anticonvulsants (e.g., levetiracetam, topiramate) may worsen behavioral domains, such as mood regulation and cognition, often already impaired in children and youth with ASD. Lastly, some commonly used anticonvulsants interact with commonly prescribed psychotropic medications in this population, mostly because, but not limited to, induction or inhibition of the cytochrome P450 isoenzymes, and as such caution is required in those experiencing polypharmacy.

Sleep

Two thirds of children with ASD report sleep difficulties (REF). Potential mechanisms include delayed melatonin peak, reduced rhythm amplitude, low ferritin, and increased periodic limb movements in sleep [21]. Such difficulties have also been reported to impact core symptom domains as well as maladaptive behaviors [22, 23]. In addition, co-occurring conditions such as anxiety disorders, gastrointestinal distress, and seizures are well known to impact sleep onset, maintenance, and overall sleep architecture. As such, management of sleep disturbance in ASD requires management of co-occurring conditions contributing to the phenotype. A toolkit outlining evaluation and treatment of sleep difficulties in children with ASD were developed by the Autism Treatment Network (ATN) (http://www.autismspeaks.org/science/resources-programs/autism-treatment-network/tools-you-can-use/sleep-tool-kit). However, if refractory, medication may be considered.

Melatonin is considered a natural health product by Health Canada. It is available in short-acting and sustained release forms, considered as "off-label" treatment of sleep disturbances in children and adolescents. Short-acting forms of melatonin are useful for sleep initiation while long-acting forms may also be useful for sleep maintenance. Its use is supported by several clinical trials and a systematic review [24]. Usual dose range in children is between 1 and 10 mg, 30–60 min prior to bedtime. There is no evidence to support melatonin use in children less than 2 years old.

Other agents typically used in this population but with weak evidence include immediate release alpha agonists, and to less extend benzodiazepines, tricyclics, and atypical antipsychotics, although the risk-to-benefit ratio for such agents should be individually and carefully considered [25].

Complementary and Alternative Medicine

When discussing biomedical treatments in ASD, it is hard to not include a discussion of complementary and alternative medicines, (CAM), defined as medical and healthcare systems, practices, and products that are not generally considered to be part of conventional medicine. The use of complementary and alternative medicine in children with ASD ranges from 27% to 88%, with 17–25% using special diets. The rate of use for CAM is higher in those with more severe ASD, those who were diagnosed earlier, and those who have comorbidities like gastrointestinal problems and seizures [26, 27]. Families turn to CAM because the etiology of ASD is not completely understood, and there is no known treatment of core symptoms. Many families would like to "try everything possible." Few CAM therapies have been proven effective or ineffective or safe in well-designed randomized controlled trials. Concern for their use also includes the competing resources for the families' finances, time, and efforts. For the sake of this chapter, we will focus on reviewing compounds or medical procedures. One may conceptualize the approach to counseling families on CAMs based on the benefits to risk evidence that may be available.

Evidence of no Benefit Secretin is a gastrointestinal hormone that inhibits motility and gastric acid release, and regulated secretion of pancreatic fluid and bicarbonate. Systematic review of several RCTs concludes that the evidence does not support its use in ASD [27].

No Evidence of Benefit, Clear Evidence of Harm Chelation is the most characteristic of those; based on a false hypothesis of heavy metal toxicity in ASD, chelation approaches were proposed to mechanistically target a potential etiology. A Cochrane review of chelation found no evidence of benefit but discussed a myriad potential side effects including hypotension, cardiac arrhythmias, and hypocalcaemia, and other metabolic disturbances, which may lead to cardiac arrest [28]. Brown et al. [29] published a case series of 3 deaths associated with chelation therapy between 2003 and 2005. The use of chelation in the context of solely ASD is strongly discouraged. Hyperbaric oxygen treatments have also been proposed, but systematic reviews have failed to identify evidence of benefit [30]. However, adverse effects associated with its use included barotrauma to the ears, reversible myopia, pulmonary barotrauma, pulmonary oxygen toxicity, and seizures. These sessions are costly, have considerable risks, and in the absence of evidence for efficacy are not recommended. There is also no basis for the prophylactic and systematic use of antimicrobial agents for core symptoms in ASD. Possible adverse effects depend on the medication used and risk of antibiotic resistance.

Possible Benefit, but Also Risk Gluten-free, casein-free diets have mixed evidence of potential benefit and clear evidence of risk (hypocalcemia, decreased Vit D, amino acids and bone density) [27]. The use of these diets in the absence of celiac disease or well documented gluten sensitivity is generally not endorsed. If engage in these diets, calcium, vitamin D, and protein intake should be monitored. There are historical accounts of vitamin B6 and magnesium used in the treatment of mental health disorders. A 2010 systematic review found inconclusive evidence of improvement in the core symptoms of ASD, and megadoses of >1000 mg/day resulted in neuropathy. ([27]; [26]). The use of high doses of these supplements are currently not recommended.

Unknown Benefit, Low Risk Two systematic reviews, a meta-analysis, and two randomized controlled trials found that there is insufficient evidence to determine if intake of omega-3 fatty acids is effective as therapy for the core symptoms of ASD [27]. Side effects include nausea, diarrhea, foul, and sticky stools. In children with ASD, pilot study doses were 1.3–1.5 g/day. Maximum dose recommended for children 8 years and younger is less than 1 g/day and for children older than 8 years is less than 3 g/day as higher doses may interfere with vitamin K metabolism. (U.S. Food and Drug Administration. *Qualified health claims: letters of enforcement discretion*) As such, routine night-dose supplementation of omega-3 fatty acids in young children with ASD is not currently recommended.

Other interventions that present low risk but do not have documented benefits include oxidative stress therapies, zinc, amino acids, digestive enzymes, vitamin C supplementation of up to 2 g/day, Acupuncture, craniosacral manipulation, and chiropractic practices. The potential utility of probiotics is currently being evaluated.

Possible Benefit, Low/Well-Defined Risk Several compounds have emerging evidence and are promising. Sulforaphane is an anti-oxidant from broccoli sprout extract. In a small clinical trial for young men with ASD (13-27 years old), its use was associated with improved behavior at a doses of 50–150 umol during treatment but behavior returned to baseline once supplementation was stopped. It was also well tolerated [31]. This remains an area of active research.

Research Directions

Despite several RCTs described above that support the use of medications for common symptoms associated with ASD, none of these medications have been discovered or understood through a translational lens. In other words, none seem to target underlying etiopathology of ASD and as such have minimal impact on challenges associated with core symptoms. However, several compounds have been identified that may engage translational targets (e.g., excitation to inhibition balance, neuropeptide systems, oxidative stress) recently revealed by genomics and system neuroscience. None of these are yet approved for use by Health Canada or any other regulatory jurisdiction.

Excitation to Inhibition (E:I) balance is a poorly defined term that is used to described several different phenomena from cellular physiology to glutamate/GABA balance measured by imaging in the human brain. To the degree that one uses the term to describe imbalance in excitatory and inhibitory signaling leading to altered neurophysiological signal-to-noise ratio [32] and differences in synaptic plasticity, this can be a useful target for pharmacologic intervention. Several genetic variants associated with ASD have been shown to lead to such E:I imbalance, such as 15q11–q13 microduplication syndrome, Shank3 deletions and fragile X syndrome (e.g., [33, 34]).

Both glutamatergic and Gabaergic agents have been considered in this category. Memantine, a noncompetitive NMDA inhibitor, which has approval in dementia, showed some promise in early studies [35], but failed to show benefit for core symptom domains in a large RCT [36]. D-cycloserine, a partial agonist of the glycine site on the NMDA receptor, showed both some early evidence for efficacy on its own, [35] but also was successful as augmentation strategy to social skills training in a phase 2 study [37]. Large RCTs have failed to confirm early enthusiasm for metabotroping glutamate receptor 5 inhibitors [38], although lessons learnt suggest that younger children may be more likely to respond,

and new RCTs in this age group are underway. Other glutamatergic agents with promise that are in trials include acamprosate, a NMDA inhibitor and $GABA_A$ agonist, and riluzole, which have presynaptic, postsynaptic glutamatergic transmission effects. Among GABAergic agonists, Arbaclofen, the R enantiomer of baclofen and a $GABA_B$ agonist have shown most promise, albeit the studies are mixed. Two large RCTs are running in both Canada and Europe to further clarify its role. A $GABA_A$a5 agonist is also in development by ROCHe, and bumetanide, a GABA modulator is in phase 3 clinical trials.

Recent evidence from system neuroscience would suggest that immune modulation, oxidative stress, and manipulation of social circuitry may also be potential targets for translational approaches. Candidate compounds targeting oxidative stress have already shown some early efficacy (e.g., Sulphoraphane, N-acetyl-cysteine, discussed above). Neuropeptides, on the other hand, have shown great promise in translational studies, but still mixed data in clinical trials, as targets for social circuitry modulation. Central release of oxytocin and its sister neuropeptide vasopressin have been implicated in social cognition and affiliation across species [39], but larger trials have had mixed results [40]. Several studies are in progress to clarify the potential for neuropeptide manipulation for therapeutic benefit. Circuitry manipulation may also be an ideal target for neuro-stimulation techniques. A recent systematic review of both tDCS and TNS studies suggests potential for benefit for both core symptom domains and executive function; however, available studies are small with high risk of bias and properly powered RCTs are currently missing [41].

Recent genomic advances have confirmed the vast biological heterogeneity in ASD, highlighting the need for personalized health care approaches. It is unlikely that one medication will benefit all individuals with ASD and identifying the various biologies that underlie the different "autisms" has become urgent. In addition, given that the developmental differences associated with ASD change with time, shaped by gene by environment interactions and compensatory mechanisms, intervention strategies have to account for heterogeneous trajectories across the lifespan. It is critical that we increase the evidence base for biomedical interventions in adults, as transitioning youth find little evidence to inform their care.

Multiple Choice Questions

1. What are the two medications approved for use in children with ASD for irritability and aggression?
   (a) Aripiprazole and risperidone
   (b) Methylphenidate and amphetamine
   (c) Risperidone and quetiapine
   (d) Clonidine and guanfacine
2. What are possible indications for the use of stimulants in ASD?
   (a) Irritability
   (b) Aggression
   (c) Hyperactivity
   (d) Anxiety
   (e) Depression
3. What is the first-line intervention for the treatment of anxiety in ASD?
   (a) ABA
   (b) CBT
   (c) SSRIs
   (d) Melatonin
   (e) Clonidine
4. What is an important consideration prior to initiation of use of stimulants?
   (a) Metabolic syndrome
   (b) Gastrointestinal symptoms
   (c) Family history of sudden death
   (d) Suicidal ideation
5. What is the acceptable dosing range of melatonin for the use in children and youth?
   (a) 1–3 mg
   (b) 3–6 mg
   (c) 6–9 mg
   (d) 1–10 mg.

Answers: 1 -a, 2 -c, 3 - b, 4 -c, 5 - d.

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Interventions in ASD: Psychosocial Interventions and Supports for ASD

21

Jessica A. Brian, Isabel M. Smith, and Katherine Stover

Learning Objectives

After reading this chapter, the reader will

1. Understand the evidence that supports a range of behavioural interventions for children and youth with ASD across development;
2. Appreciate the evidence gaps and areas in which further research is needed;
3. Appreciate the need for matching individual and family needs with available treatment approaches to foster personal and family quality of life;
4. Understand the important role of parents in interventions for children and youth with ASD;
5. Gain a comprehensive understanding of intervention approaches for toddlers and very young children with emerging or confirmed ASD;
6. Learn about group-based social skills programs for youth with ASD;
7. Become familiar with a range of educational supports for secondary and post-secondary students with ASD;
8. Understand the main evidence-based approach to mental health treatment for children and youth with ASD;
9. Gain insight into the application of behavioural strategies, including positive behaviour supports, to manage interfering behaviour in children and youth with ASD.

Highlights

- Behavioural interventions for individuals with ASD encompass a range of developmental, educational, and mental health treatment approaches.
- The bulk of current evidence supports the efficacy and effectiveness of interventions based on the principles of applied behaviour analysis (i.e. ABA-based programs, including cognitive-behavioural therapy, CBT) within a developmental framework.
- Parent involvement is a key component of many successful treatment approaches.
- Many interventions developed specifically for the toddler years are delivered by parents, supported through coaching.
- Group-based social skills programs and peer-mediated approaches may be particularly effective for helping school-aged children to develop positive peer interactions.
- Consultative models for academic and school-based interventions hold promise for teacher-level training and support that can extend beyond an individual student.

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- CBT interventions are effective for reducing anxiety symptoms in children and youth with ASD.
- The provision of positive behaviour supports, and parent training in use of behavioural strategies, can be effective in reducing disruptive behaviour that interferes with participation and learning.

Introduction

Interventions for individuals with autism spectrum disorder[1] (hereafter ASD or autism) take a variety of forms, often being divided into pharmacological and non-pharmacological, or psychosocial, approaches. Broadly speaking, psychosocial interventions encompass a range of behavioural, developmental, and educational models, as well as mental health treatments (e.g. cognitive-behavioural therapy, CBT). The greatest accumulation of evidence supports the efficacy of approaches based on the science of learning and behaviour (i.e. applied behaviour analysis [ABA] and CBT models). The overarching objectives of behavioural and educational programs are to facilitate learning and acquisition of skills that meet individual and family needs and priorities, to reduce, replace, or manage behaviour that interferes with successful participation in learning, community, and social opportunities, and thereby to enhance the quality of life for individuals with ASD and their family members.

Intervention targets vary across individuals and over development. In the early years, the focus is typically on the acquisition of language and enhancement of social communication, social engagement, and play skills. The past decade has seen a groundswell of intervention models developed and evaluated specifically for use with toddlers. By the preschool and early school years, program priorities are increasingly variable across children, due to greater heterogeneity in ASD presentation. For example, some children present with significant intellectual disability and limited communication skills whereas others may have strong verbal language skills with more subtle social and behavioural differences. Throughout the school and post-secondary years, children and youth with ASD may need a variety of academic, social, and mental health supports in order to foster meaningful and satisfying community engagement and thriving. Moreover, support needs do not end with adulthood.

[1]In this chapter we use person-first language (e.g., "individuals with autism") to reflect the most common usage in the current literature. We also note that opinions on this issue vary and that a preference for identity-first language (e.g., "autistic individuals") is emerging in some contexts.

Although beyond the scope of this book, the sobering outcomes in adulthood for many autistic individuals bear highlighting, with high levels of unemployment and other economic disadvantages, as well as low levels of social support [1]. Reviews of research regarding services for adults with ASD highlight an anticipated increase in the already high need for services, accompanied by serious gaps in knowledge of effective practices [2, 3], recognizing that the needs of adults with ASD vary considerably across individuals and over time [4]. A scoping review of 17 qualitative studies highlighted the key barriers and facilitators of successful transition to adulthood, underscoring the importance of individualized supports, person-centred planning, environmental modification, gradual transitions, and information-sharing and collaboration [5].

In this chapter, we highlight behavioural and cognitive-behavioural interventions that address general learning and developmental targets, social skills, anxiety symptoms, and management of disruptive or interfering behaviour, from toddlerhood into adolescence. We begin with a discussion of ABA-based approaches, with a focus on their application in the preschool and early school years. Next, we focus on emerging treatment models for toddlers with ASD, many of which are parent mediated. In addressing the unique needs of school-aged children and youth, we highlight group-based and peer-mediated social skills programs, educational supports, and we touch on cognitive-behavioural therapy (CBT) for treating common conditions such as comorbid anxiety problems. Finally, this chapter concludes with discussion of intervention approaches for disruptive behaviour challenges in individuals with ASD.

Behavioural (ABA-Based) Intervention

In general, interventions demonstrated to be effective for children with ASD are based on the empirically derived principles of learning, via strategies and procedures of ABA [6, 7]. The field of ABA encompasses the application of procedures that either strengthen (desirable, adaptive, socially meaningful) or decrease (interfering, or maladaptive) behaviours. A foundational construct in ABA is the 'three-term contingency' (antecedent—behaviour—consequence, or A-B-C). Analysis of behaviour within this construct, and manipulation of factors within this contingency (e.g. implementing antecedent conditions that encourage a particular behaviour, or use of reinforcing consequences, which lead to future increases in the preceding behaviour) form the basis of ABA procedures. ABA-based teaching strategies vary, from highly controlled applications (e.g. discrete trial teaching, DTT, which involves repetition of precise instructional 'trials' within a quiet, one-to-one setting), to procedures carried out within naturalistic contexts that capitalize on teach-

ing opportunities occurring within daily activities in the child's typical environments such as the home, playground, childcare setting, preschool, or school.

Some evidence supports both controlled and more naturalistic ABA approaches [8], but a critical outstanding question is which ABA procedures and programs are best suited for which learning targets, for which learners, at which ages, and in which contexts. One critical challenge in the field of ABA, as it pertains to treatment for children with ASD, is that the bulk of the high-quality evidence comes from single-subject design (SSD) studies [7]. Although this rigorous research design has demonstrated the efficacy of many specific ABA-based procedures for children and youth with ASD [7, 9], few group-design studies have been employed to establish the overall efficacy of programs that combine these procedures (e.g. see [10, 11], for reviews). Moreover, apart from some notable exceptions (e.g [12, 13].), the effectiveness of service delivery models that implement treatment at the community level remains to be established.

ABA-Based Intervention in the Preschool and Early School Years

Although ABA techniques can be implemented at any age, specialized ABA-based intervention programs are available for preschoolers with ASD in most jurisdictions across North America. Early specialized intervention programs are often characterized as either 'comprehensive', if multiple developmental domains are addressed within the program, or 'targeted', if the targets of treatment are more focused [7]. Many comprehensive programs for young children have evolved from the model developed by Lovaas at the University of California at Los Angeles (UCLA; [14]). These are often referred to as 'early intensive behavioural intervention' (EIBI) programs.

Systematic reviews and meta-analyses have demonstrated that EIBI programs based on the UCLA model yield improvements in cognitive, language, and adaptive functioning for many children with ASD (see [6, 10]). The factor most strongly associated with the best outcomes appears to be the quality of the program, as evidenced by expert supervision and formal monitoring of treatment integrity [15], and the inclusion of parent involvement in decision making [10]. Although considerable evidence supports the efficacy of EIBI programs for preschoolers with ASD, the quality of the evidence is considered low when judged by the standards required for medical treatments (e.g. randomized control trials; RCTs; [6, 10, 11]). Inherent barriers to conducting RCTs of complex, multi-component, and long-term (i.e. often two- to four-year) programs mean that few well-controlled efficacy trials exist of such comprehensive programs [16], and direct comparisons of different approaches are rare (although three recent exceptions are discussed below). Nevertheless, some evidence on the *effectiveness* of early intensive ABA-based intervention programs for preschoolers with ASD comes from community-based programs. Findings from Canada [12, 17–19] and Sweden [20] demonstrate that, for many children, outcomes can be enhanced through wide-scale implementation of well-supervised ABA-based intervention programs.

Three studies have directly compared the efficacy of different high-quality comprehensive preschool programs for children with ASD [8, 13, 21]. Boyd et al. [21] used a quasi-experimental design to compare outcomes from two well-known intervention programs for preschoolers with ASD (Learning Experiences and Alternative Program for Preschoolers and their Parents, LEAP; and Treatment and Education for Autistic and Communication Handicapped, TEACCH) and a control program that did not declare a specific treatment model. Smith et al. [13] used a parallel cohort design to systemically compare outcomes from a naturalistic ABA-based approach (Pivotal Response Treatment, PRT; [22]) to more traditional EIBI that included DTT, delivered through community providers across two Atlantic provinces in Canada. Finally, an RCT design was employed to systematically compare outcomes from a comprehensive DTT-based program to those of a naturalistic approach (Early Start Denver Model, ESDM; [8]). In all three studies, treatment gains were comparable across intervention types. While conclusions drawn from quasi-experimental and parallel cohort designs are complicated by differences in ascertainment, case definition, characteristics of the children, and intensity of intervention, they are echoed by the RCT findings. Specifically, these studies support the notion that high-quality programs share key elements that may be more important than the program-specific characteristics that distinguish them, echoing a suggestion with a long history [23]. The identification of key-active ingredients remains a priority in understanding the mechanisms of change associated with multi-component ASD interventions [24, 25]. Moreover, policy-related considerations (e.g. cost, training, and certification requirements) remain under-studied (see [26, 27], for notable exceptions).

Parent Involvement in ABA-Based Programs

One common element of effective programs entails involvement of parents in the intervention (e.g [8, 13]). Two decades ago, National Research Council [28] guidance identified the involvement of parents as a key element in effective early intervention programs for ASD. The nature of that involvement varies across programs, from parents acting as therapists in structured DTT sessions [29], to programs that support parents' use of naturalistic ABA-based methods such as PRT [13, 22], which can be readily learned by parents and incorporated into everyday routines [30, 31]. Parent involve-

ment, particularly in naturalistic applications, maximizes the child's ability to use skills across contexts (i.e. 'generalization'; [32]), thus, enabling children to practice and apply skills throughout daily life. Across programs, various methods are used to train parents, including didactic (psychoeducational) information sharing, modelling, reflection, video feedback, and direct in-the-moment coaching, among others (discussed further below). Training can occur in group formats [33] or individually [34]. Moreover, parent involvement can serve as an adjunct to more intensive programming or may stand alone. Unfortunately, the terms 'parent-mediated' and 'parent-implemented' are used somewhat interchangeably in the literature, to reflect these approaches, leading to some confusion [35]. An initial meta-analytic review demonstrated the promise of parent-mediated interventions for young children with ASD (aged 1 year to 6–11; [36]). However, that review combined evidence from approaches in which parent involvement complemented more intensive programming delivered by therapists (e.g [37].) with those that were exclusively delivered by parents (e.g [38].), muddying the conclusions that could be drawn. Evidence supporting early parental involvement in intervention has grown substantially over the past decade, with increasing focus on programs that are exclusively parent delivered (or parent implemented), often for use in the toddler years, as described in detail below. At the end of this chapter, we discuss the important role of parent training in managing disruptive behaviour.

Naturalistic Treatment Approaches for Young Children

Many ABA-based programs operate within a child's typical (home or childcare) environment, and prioritize naturally related cues and responses (e.g. use of reinforcing consequences that are naturally tied to the child's responses, such as giving a toy when the child asks for it). A distinction has been made in the literature between naturalistic intervention models that build explicitly upon learning theory and employ ABA-based strategies in naturalistic contexts within a developmental framework ('naturalistic developmental behavioural intervention'', NDBI, approaches; [39]) and those that are rooted primarily in developmental theory, with less emphasis on ABA methods (often referred to as developmental, or 'developmental social pragmatic', DSP, models; [40, 41]). However, despite efforts to categorise programs according to this taxonomy (e.g [11, 40, 42].), the distinctions are not consistently articulated and many characteristics overlap [40]. Nevertheless, the following discussion provides an overview of the evidence for models situated by their developers within one of these approaches.

Naturalistic Developmental Behavioural Intervention (NDBI)

As the field of autism intervention has evolved, the application of ABA-based strategies in contexts that are more natural for young children has gained appeal, with a focus on applying these strategies within a developmental framework (i.e. capitalizing on knowledge of typical developmental relations between the emergence of behaviours such as joint attention or imitation and language). As such, programs taking this stance have been characterized as NDBIs [39]. As with other models, NDBI approaches aim to incorporate parents so that learning can be supported in the child's home, in an effort to promote generalization and application of learned skills. NDBI models can be primarily therapist delivered (with parent training as an adjunct) or exclusively parent delivered, or hybrid (combined therapist and parent delivered). They also vary considerably with respect to program composition (comprehensive or targeted), duration, location (e.g. home, preschool, both), and specific targets. All NDBI models incorporate naturalistic ABA-based strategies within a developmental framework, using manualized procedures and rigorous training protocols, and emphasize the importance of treatment integrity [39].

Pivotal Response Treatment [22] is a prominent NDBI approach that targets the core areas of motivation, self-initiation, self-management, and responding to multiple cues based on the premise that targeting core (or 'pivotal') learning domains will lead to behavioural gains across a wide array of learning needs [43–45]. Despite usually delivered primarily by therapists, the inclusion of a parent-training component is paramount in PRT [22, 45, 46]. Indeed, RCT evidence supports the efficacy of PRT when delivered exclusively by parents [47]. A systematic review of five RCTs concluded that PRT has significant positive effects on children's expressive language skills. However, summary conclusions about the effect of PRT on other skills have been limited due to methodological challenges and the small number of studies [48]. These findings have since been bolstered by an RCT of a 6-month 'packaged' PRT model for toddlers that placed increased emphasis on parent training to support therapist-delivered program goals [31]. Following 24 weeks of intervention, children receiving PRT made significant gains in social-communication skills, compared to waitlist controls.

A meta-analysis of 27 group-design NDBI studies for young children (mean age under 6 years) showing symptoms of or diagnosed with ASD revealed large positive effects for social engagement and cognitive development. Smaller but significant effects emerged for expressive language, ASD symptoms, and play ([49]; note that we describe some of these studies below in the section on toddlers). NDBIs stand out as among the models best sup-

ported by high-quality evidence when evaluated alongside other intervention modalities (i.e. behavioural, developmental, sensory, animal-assisted, and technology-based approaches). In a recent systematic review and meta-analysis of early intervention group-design studies in young children with ASD, Sandbank et al. [11] concluded, 'by far, NDBIs have emerged as the intervention type most supported by evidence from RCTs' (p.17), with over three-quarters of all published NDBI studies employing an RCT design to evaluate outcomes. This represents a significant advance, made possible in part by the fact that NDBI programs are usually shorter (often time-limited) than comprehensive EIBI programs (which typically last more than one year), and thus, more amenable to RCT designs (e.g. less prone to contamination).

Developmental and Social-Pragmatic Intervention Models

Developmental Social-Pragmatic (or Developmental) approaches focus on integrating typical developmental sequences into intervention techniques, with social communication as a primary target [40, 41]. Such approaches share elements with the NDBI models, with perhaps more similarities than differences [40]. Evidence to support the efficacy of DSP models varies depending on the specific approach. One of the most widely known DSP interventions (Developmental, Individual differences, Relationship based; DIR/Floortime; [50]) has been the subject of a systematic review that concluded that, despite some suggestion of improvements in social-emotional development, the low methodological quality (e.g. limited RCTs, no independent replication) and rigour (e.g. selection bias) of studies assessing this model limit conclusions about its efficacy [51]. Similarly, a parent-mediated DSP program used widely by speech–language pathologists in some Canadian jurisdictions was evaluated through a well-powered RCT which yielded no significant overall gains in children's communication skills, nor in parental responsiveness to children's cues [38]. Whether these results were due to limitations in the selected outcome measures, the techniques themselves, or in parents' ability to use them effectively, remains to be evaluated, as parents' fidelity of implementation was not reported.

A recent systematic review of ten interventions identified by the review authors as DSP approaches concluded that consistent gains were reported in foundational social-communication skills. These included children's attention, focusing on faces, responding to bids for joint attention, engaging in reciprocal interactions, and initiating communication. However, the heterogeneous study designs, methods, and outcome measures precluded the use of meta-analytic techniques, thereby limiting the opportunity for quantitative substantiation of the efficacy of these interventions [42]. Further, the interventions were found to have varying effects on children's language skills. Some studies showed small-to-moderate positive effects in children's receptive (but not expressive) language, whereas others showed no significant differences on standardized language tests, but moderate-to-large positive effects on children's language use based on analysis of videotaped natural interactions. In their comprehensive systemic review and meta-analysis of group-design ASD intervention studies more broadly, Sandbank et al. [11] evaluated 14 models designated as 'developmental' (which overlapped with those designated as DSP by [42]), ten of which were tested in RCTs. Despite some evidence that developmental models may be efficacious for social-communication skill development, the authors warned that, when studies with high levels of bias associated with over-reliance on caregiver report and high rates of attrition were removed from analyses, too few studies remained with which to determine summary effects.

Given the lack of consensus on how best to classify programs as NDBI vs. DSP, we collapse the evidence from both in the following section that focuses on intervention programs specifically designed for children in the toddler years.

Intervention Approaches for Toddlers

As the field has made gains in early detection and diagnosis of ASD, the need for intervention approaches that are sensitive to the unique needs of toddlers has become paramount [52]. Over the past decade, several programs have been developed and evaluated specifically for use with toddlers. These programs generally focus on one or more commonly accepted core domains for toddlers with ASD, namely, social communication, social attention, engagement, and play.

Toddler treatment approaches can be therapist or parent delivered (or hybrid). One of the most studied approaches, ESDM, has been evaluated through well-powered RCTs. An initial study yielded positive findings based on a hybrid model in which toddlers received an average of approximately 30 h of intervention (15 therapist-delivered, plus 16 parent-delivered hours) per week, over a two-year period [37]. Results revealed significant gains in IQ, adaptive behaviour, and changes in diagnostic categorization in toddlers who received ESDM therapy, when compared to a community group receiving treatment-as-usual. A subsequent multi-site RCT [8] partially replicated the original findings, yielding evidence of treatment advantage at two (of three) study sites on a combined language measure, but not on IQ, autism severity, or adaptive behaviour, following 2 years of intervention. This partial replication highlights the complexity of evaluating such comprehensive programs, and again points to the importance of identifying the core elements within multi-component interventions that have therapeutic benefit (i.e. the 'active ingredients'; [25]).

Although comprehensive therapist-led programs may hold promise for toddlers with ASD, many jurisdictions resist implementing them based on costs. The past decade has seen exponential growth in models that leverage parents and other primary caregivers as the mediators of change (i.e. parent-delivered or parent-implemented programs). Expanding the availability of programs that can be delivered by parents can result in increased access to intervention and may be cost efficient. Moreover, such interventions may lead to improved generalization and maintenance of acquired skills [45], and may positively influence parent–child interactions with potential for long-term collateral gains (e.g. by enhancing parental synchrony or responsiveness; e.g. [53].).

'Packaged' Parent-Implemented Interventions for Toddlers

Involvement of parents as the primary, or even sole, providers of the intervention has gained traction over recent years. The majority of this work has focused on 'packaged', time-limited programs for use with toddlers or very young children with ASD. Such models are sometimes described as 'parent-mediated', to reflect the premise that parents learn skills that are intended to effect (parent-mediated) change in the child. However, hybrid models are also often described in the literature as 'parent-mediated' (e.g [36].), leading to some confusion. Terms like 'parent-implemented' [32] or 'parent-delivered' may more precisely describe approaches in which parents are the sole implementers of the enhancing techniques, but these terms fail to capture the putative mechanism of (parent-mediated) change in the child. Some models, such as ESDM (described above), originally developed to be a comprehensive, hybrid approach [37], have been modified for sole delivery by parents [54, 55]. However, when ESDM was adapted for full parent delivery in a brief (12-week; P-ESDM) model, no advantage emerged for the treatment group over community treatment-as-usual controls [54]. Several confounding factors might explain these negative findings, including a high dosage of intervention in the control group, equivalent gains in the use of ESDM strategies for both groups (again highlighting the issue of common features across programs), and the substantially shorter duration of the parent-delivered (12 weeks) than the hybrid model (i.e. 2 years).

Several other toddler-focused treatment models have been designed specifically as parent-delivered approaches from their inception, most being described as either NDBI or DSP models. Many such programs have now been delivered as time-limited treatment packages and evaluated in RCTs, yielding a range of positive outcomes in key developmental domains including social attention, communication, parental responsiveness, and shared positive affect (e.g [56–62]) following relatively short periods (i.e. 6–12 months). Other important outcomes have emerged, such as increased parent-reported self-efficacy and empowerment [62], parental responsiveness, and parent–child synchrony (e.g [53, 56, 63]). Notably, however, some RCTs in this area have failed to yield positive effects for the primary child-level outcomes (e.g. [38, 53, 54]), highlighting that work remains to be done with respect to identifying core treatment elements, targets, and outcome metrics, as well as the profiles of children who benefit differentially from particular programs. Finally, differences in the methods used to teach intervention techniques to parents may play a key role in variability across studies. Based on an interdisciplinary meta-analysis, Sone et al. [64] conclude that active coaching appears to be the best way to promote adult learning within the context of parent-delivered early intervention.

The majority of the work described above has focused on evaluation of short-term gains in children's and parents' behaviours or skills, but of course, longer term impacts are the ultimate objective. By arming parents with a set of strategies that can be used within typical daily interactions, the premise is that, even following a relatively short 'training' period, parents will continue to use those enhancing strategies with their children, thus influencing longer term development. The first parent-mediated treatment program for toddlers and preschool-aged children to show long-term benefits following a relatively brief intervention (i.e. 12 months of parent training) was the Preschool Autism Communication Trial (PACT; [56, 65]) from the United Kingdom. An initial RCT showed no treatment effect for the primary outcome, but revealed improved parental synchrony, child initiations, and parent–child shared attention in children aged 2 to 4–11. Notably, a six-year follow-up study did yield a significant treatment effect for the primary outcome (i.e. reduced core ASD symptoms) in the group who had received PACT as preschoolers [65]. These findings point to the potential downstream impacts of programs that enhance parent–child interaction very early in development.

In addition to gains in young children's social-communication skills, benefits of parent-implemented intervention include the adoption and integration of strategies into daily routines [32] and the subsequent potential increased 'dose' of intervention [66], the use of fewer system resources (e.g. therapist hours, travel), and thus, greater cost efficiency, when compared to therapist-implemented interventions [38, 67], and the potential to increase parents' sense of efficacy and empowerment [32, 66].

Group-Based Social Skills Interventions

As children enter the school system, an important shift in their social world occurs, from a focus on the parent–child relationship toward an increased focus on peer interaction. One of the primary areas of need for children and youth with

ASD is the enhancement of skills to engage in flexible and satisfying social interactions, particularly with peers. In addition to ABA-based and classroom social skills supports (described below), group-based social skills programs can be effective in meeting this need.

Group social skills programs for children and youth with ASD are designed for those with relatively stronger language skills, which are often felt to be required for successful engagement and learning in such programs. Some evidence supporting group social skills programs comes from a meta-analysis of eight RCTs, which demonstrated large positive effects on social communication and moderate effects for social skills (e.g. cooperation, responsibility), as measured largely by parent or self-report [68]. These findings supported those of an earlier large meta-analysis of 115 studies that used single-subject research designs, which concluded that social skills programs are beneficial for children and youth, including those with ASD [69]. More recently, a historical review of single-subject design studies [70] identified directions for future investigation in this field, including the need to investigate core components of social competence and factors that influence fidelity of implementation, and highlights the importance of further culturally and linguistically relevant research in natural contexts [70]. Moreover, successful application of social skills beyond the learning context is a major challenge for individuals with ASD, so it is critical that social skills programs explicitly address generalization to other settings.

Although treatment parameters are not well described in many studies, converging evidence on the Program for the Education and Enrichment of Relational Skills (PEERS; [71]) reveals that the social skills of school-aged children and adolescents [72–74] as well as young adults [75] with ASD can improve with participation in this manualized parent−/caregiver-assisted program. Some evidence suggests generalized effects such as increased frequency of social get-togethers [71, 74].

A promising approach to teaching social (and other) skills is to engage typically developing children and youth as intervention agents for their peers with ASD. A review of 56 such peer-mediated interventions, evaluated through single-subject research design studies, concluded that they produced positive outcomes for a range of behaviours, including social interactions, with some evidence of generalization [76]. This has been echoed in programs based specifically on training peers in the use of PRT-based strategies [77], pointing to the promise of such interventions within inclusive settings. The vast majority of evidence in this area comes from single-subject design studies, but some group-based or pre-post designs have been conducted. Five such studies were reviewed by Chang and Locke [78], four of which were RCTs, and all included a specified training component with teacher-nominated peers. This systematic review concludes that peer-mediated interventions can result in significant gains in social skills for children with ASD. Indeed, one RCT included in the review demonstrated that a 12-week peer-mediated social skills intervention resulted in greater gains in terms of meaningful classroom engagement than an intervention involving adult-directed teaching [79]. The authors highlight current limitations in the literature, including considerable variability with respect to blinded evaluation, fidelity measurement, sample size, and use of formal statistical analyses. Further, they note that most evidence comes from children with ASD who have average to above-average cognitive functioning and thus may not generalize beyond that subset of children. Only one study specifically focused on preschoolers (aged 3–4 years), but this study was limited by a very small sample (i.e. 5, only 3 of whom received intervention), absence of fidelity measurement, and no formal statistical tests of outcomes.

Educational and Other School-Based Supports

Inclusive education is a guiding principle in most North American jurisdictions. Many school-aged children with ASD, regardless of classroom placement, will benefit from individual educational plans (IEPs) or individual program plans (IPPs). It is important to ensure that both academic and non-academic goals are incorporated into these plans to ensure that social, emotional, and recreational learning needs are not overlooked [80]. These plans may resemble programming for preschoolers with ASD, particularly among students with intellectual disability (ID) or more complex learning needs. However, many students with ASD will be served within inclusive settings with relatively minor modifications to the regular curriculum.

Academic and Learning Supports

Contrary to some claims, no specific profile of cognitive skills or learning profile is consistently associated with ASD. For instance, although a so-called 'nonverbal advantage' (i.e. lower verbal cognitive skills relative to nonverbal performance) may be common, the opposite pattern may also occur, with students displaying stronger verbal proficiency [81]; many students will show no significant discrepancies between verbal and nonverbal abilities. Other aspects of learning and cognitive challenges also vary considerably across students with ASD, such as processing speed and executive functions (e.g. attentional control, working memory, planning/organization; [82]), and motivational factors may play a key role in learning [83].

The reported prevalence of intellectual disability (ID) as a co-occurring condition in individuals with ASD has changed as the definition of ASD has evolved in recent decades. Recent population-based estimates range from 12% to about

40% [84]. The DSM-5 [85] provides a framework for formal recognition of conditions that may co-occur with ASD, including ID and specific learning disorders.

Given these considerations regarding intellectual ability and learning challenges, academic programming should be responsive to each individual's profile (i.e. cognitive and academic strengths and weaknesses) defined in a formal neuropsychological/psychoeducational assessment. To optimize emotional well-being and self-confidence, academic programming should focus on fostering the student's strengths and interests rather than focusing exclusively on the remediation of areas of weakness. Other basic educational principles include the importance of adjusting teaching methods to accommodate characteristics commonly associated with ASD, such as difficulties with generalization and the application of knowledge in real-world contexts [80].

Consultative models may allow for teacher-level training and support that can extend beyond a particular student. The Collaborative Model for Promoting Competence and Success (COMPASS; [86]), is a manualized consultative model with demonstrated efficacy. Two RCTs of this model have demonstrated improved attainment of IEP goals in preschool and elementary school settings [87, 88] through both in-person and video-conferencing consultation.

Other School-Based Interventions

Ideally, educational programming for students with ASD will address social and adaptive behaviour needs (including those related to social inclusion and personal safety) in addition to academic goals. Guidelines for educational best practices that address all of these areas are available from two major initiatives in the US ([7, 89]; also see [90]). In addition to peer-mediated social skills supports described above, other school-based social skills interventions can have positive impacts on students' social behaviours, although a recent review [91] concludes that such approaches tend to be resource-intensive (i.e. typically delivered by research teams or teaching assistants outside of the classroom), raising important questions about their sustainability.

Unfortunately, the high prevalence of bullying of children and youth with ASD – recently estimated at 44% of adolescents based on a systematic review and meta-analysis of 17 studies [92]—has made this form of victimization a critical consideration in educational contexts for students with ASD and their peers. Specific effectiveness of mainstream bullying prevention programs for youth with ASD is unknown. Some have argued that bullying prevention and monitoring strategies should be part of the student's IEP as well as a school-wide effort [93]. Maiano et al. [92] highlighted the need for multi-level comprehensive approaches to bullying interventions that focus not only on the child with ASD, but that also provide training for peers, teachers and support staff.

Post-Secondary Education

Educational opportunities and supports for older adolescents and young adults have gained attention as increasing numbers of individuals with ASD graduate from secondary school and pursue post-secondary education. Limited evidence exists to support specific educational practices in this age range, but work with individuals with other developmental and learning disorders may provide some direction. Key factors include timely, individualized assessment of strengths and interests, and preparation for realistic post-secondary options. Transition planning ideally begins in early adolescence and includes teaching skills related to self-determination (i.e. development of skills, attitudes and beliefs enabling control over one's own life; [94]) as part of the educational curriculum. Teaching such skills may be particularly essential yet uniquely challenging for individuals with ASD, for whom both sense of identity and ability to self-evaluate may be reduced relative to their peers [95]. Moreover, recent work reveals that social challenges and bullying can persist into this phase of life [96], highlighting the importance of providing social and mental health supports in addition to any academic accommodations that may be indicated.

Consultation models can be an effective way to support post-secondary transitions for youth with ASD. The COMPASS model, originally developed and evaluated for use with preschool and elementary students (described above), has been successfully adapted for use with transition-age youth in the US public education system [97]. A recent RCT involving 20 special education teachers demonstrated that students receiving the COMPASS model attained two thirds of their IEP transition goals, compared to fewer than 20% in a control condition [97]. This study highlights the important role that evidence-based consultative services can play in supporting post-secondary transition for youth with ASD.

Mental Health Supports

Children and youth with ASD experience high levels of mental health difficulties, especially anxiety [98], with reports as high as 79% [99]. Some evidence suggests that higher rates of anxiety may be associated with older age ([100], but others do not report age effects; see [101, 102]), milder ASD symptoms ([103, 104], but see [105]), and higher cognitive abilities ([101, 104], but see [98, 106], for contrary findings).

Psychosocial interventions such as CBT have gained prominence in treating affective disorders, and particularly anxiety, in ASD over the past decade (e.g [107].), with some programs designed or adapted specifically for children and youth with ASD. Group and individual CBT programs have been shown to yield improvements in anxiety symptoms in the context of controlled laboratory settings using RCT and single-case designs for children and youth with ASD aged 7–17 (see [107], for a comprehensive review). Recent efforts have demonstrated the feasibility and effectiveness of CBT interventions when implemented in community clinical settings [108–110], with emerging evidence to support their use in schools [111, 112]. As with most behavioural interventions, questions remain as to which child and family variables have the greatest impact on response to treatment, but such programs have demonstrated group-level effects in anxiety reduction across several studies and intervention settings as described by Weston et al. [107].

Behavioural Interventions for Disruptive Behaviour Challenges

Challenges with communication, emotion regulation, and behavioural flexibility, together with high anxiety and possibly atypical reactions to sensory experiences, can all lead to disruptive behaviour [28] in individuals with ASD. These may be manifested as tantrums, aggression, property destruction or self-injurious behaviour, and need to be addressed when these behaviours interfere with an individual's well-being and ability to access and participate successfully in community, social, and academic opportunities.

Positive behaviour (intervention and) support (PBS/PBIS; [113]) is an approach that addresses interfering behaviour problems that emphasizes manipulating antecedents ('A' in the A–B–C contingency) to reduce the likelihood of disruptive behaviour occurring, rather than only by providing consequences for undesirable behaviour. 'Functional behaviour assessment' or 'functional analysis' of behaviour [114] is a process of analysing the antecedent conditions and the reinforcing consequences that contribute to a disruptive behaviour. For example, a child who frequently hits his chin with his fist may be doing so for a variety of reasons. He may do this every time, an academic demand is placed on him because adults have reliably removed the requirement to comply with a difficult task whenever he has done this in the past (i.e. he has learned that this behaviour results in the removal of task demands). On the other hand, he may hit his chin in response to an unidentified toothache, or as a stress reaction when the classroom gets too loud. Only by understanding the *function* of the behaviour can a tailored approach be developed to address the problem (e.g. teaching the student to ask for help; teaching the skills required to master the task; treating a physical problem; and changing the sensory environment). Implementation of timely and appropriate behavioural interventions may mitigate the need for psychoactive medications, which are all too commonly used for managing disruptive behaviour in individuals with ASD [115].

A recent systematic review of eight RCTs (all using manualized interventions) reveals that, among school-aged children with ASD, parent training [116] in behavioural strategies can be effective in promoting positive behaviour and managing disruptive behaviour. School-based interventions have also been shown to be effective in a recent large systematic review of single-subject design studies [117]. However, most of this evidence comes from segregated settings, and RCT evidence remains scant. Recent work has demonstrated the efficacy of a packaged program aimed at reducing challenging behaviour in school-aged children and youth with ASD, designed specifically for delivery through publicly funded mental health services (AIM-HI; [118]). A recent cluster randomized clinical trial in a large community sample demonstrated the efficacy of this approach, with significant reductions in disruptive behaviours for those receiving the AIM-HI protocol for 18 months, relative to controls receiving usual treatment [118].

Conclusions

Intervention approaches for children and youth with ASD take a variety of forms, with the bulk of evidence supporting the efficacy and effectiveness of programs that are based on the principles of learning (i.e. ABA- and CBT-based models). ABA-based approaches can be applied in preschool and school settings, and can involve parent training that allows children to practice skills at home – several models developed specifically for the toddler years highlight the critical role that parents can play in mediating learning in their very young children, with emerging evidence of downstream positive effects. Supports for school-age children and youth include classroom behavioural and academic interventions, peer-mediated and/or group social skills training, and therapy for emerging mental health challenges. Disruptive behaviour problems that interfere with learning or participation, are often best addressed using ABA-based approaches, with evidence supporting the important role of parent training to manage disruptive behaviour across contexts.

Efforts to identify predictors of treatment response in children receiving comprehensive ABA-based interventions have yielded evidence that factors such as age and developmental level contribute, but there tend to be complex interactions between these and other important factors ([119]; and [120], for reviews). Many questions remain about the role of other variables associated with the intervention (e.g. inter-

vention type, delivery method, setting, 'dose', duration; [8]), as well as child and family factors (e.g. parents' stress and coping, and socio-economic factors, which are particularly salient for parent-mediated programs; see [121]). Current best practices highlight the importance of implementing approaches that are supported by sound research evidence and that address developmentally relevant and socially meaningful treatment targets, informed by knowledge of the child, the family, and specific treatment parameters [119, 122]. Ultimately, selection of therapeutic approaches and programs involves maximizing 'goodness of fit' between a program and the child's or youth's current developmental, learning, social, and mental health needs and strengths, and their own and their family's preferences and priorities.

Multiple Choice Questions

1. What are the components of the 'three-term contingency' in applied behaviour analysis?
   (a) approach – avoidance – connection,
   (b) antecedent – behaviour – consequence,
   (c) fright – flight – freeze,
   (d) affect – behaviour – communication.
2. Parent-implemented intervention for toddlers with emerging ASD can yield gains in children's social-communication skills and may allow for:
   (a) the adoption of strategies into daily routines,
   (b) lower use of system resources,
   (c) parental empowerment,
   (d) all of the above.
3. Group social skills programs for children and youth with ASD are:
   (a) designed for individuals with relatively stronger language skills,
   (b) designed for individuals with limited language skills,
   (c) only effective in adolescence,
   (d) only effective in early childhood.
4. Recent estimates of the prevalence of bullying of youth with ASD:
   (a) 10–20%
   (b) 30–50%
   (c) over 70%,
   (d) under 10%.
5. Educational programming for students with ASD should address:
   (a) academic skills only,
   (b) academic skills, social, and adaptive needs,
   (c) social skills and life skills only,
   (d) adaptive and life skills only.
6. Evidence supporting the effectiveness of anxiety-reduction programs using cognitive-behavioural therapy comes from:
   (a) randomized controlled trials in laboratory settings,
   (b) single-case research design studies,
   (c) community clinical settings,
   (d) school settings,
   (e) all of the above.
7. Positive behaviour supports address interfering behaviour problems by focusing on:
   (a) introduction of consequences,
   (b) punishment of disruptive behaviour,
   (c) active ignoring of disruptive behaviour,
   (d) manipulation of antecedent events or conditions.

Multiple Choice Questions –ANSWERS!

1. **B**
2. **D**
3. **A**
4. **B**
5. **B**
6. **E**
7. **D**

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Part III

Fetal Alcohol Spectrum Disorder

Clinical Perspectives on the Diagnostic Assessment of Individuals with FASD

22

Ana C. Hanlon-Dearman and Sally Longstaffe

Learning Objectives

Using a case study, this chapter will highlight clinical pearls in the medical assessment of FASD with the following objectives:

1. Understand pertinent considerations in taking a sensitive alcohol history
2. Review common symptoms of FASD in infancy, preschool, school age, adolescence/adulthood
3. Describe the 3 sentinel facial features that may be associated with FASD
4. Identify the domains of brain function to be assessed in FASD
5. Discuss differential diagnosis of FASD
6. Apply principles of diagnostic assessment to a case study

Chapter Highlights

- Factors associated with prenatal alcohol use need to be understood and contextualized to reduce stigma and improve prevention efforts; prenatal alcohol confirmation needs to be approached in a sensitive and non-judgmental manner
- It is important to recognize the symptoms of FASD across the lifespan, from infancy to adulthood; symptoms may be overlooked at any age, particularly in the absence of dysmorphology
- Individuals with FASD may have complex and adverse life experiences such as trauma which also contribute to their presentations and should be considered in differential diagnosis
- Differential diagnosis of FASD includes many genetic disorders; medical assessment should carefully consider dysmorphology and behavioural phenotype and consider further genetic testing, particularly in the presence of significant cognitive deficits and family history of intellectual disability
- Prenatal alcohol exposure may affect multiple organ systems; a thorough review of systems and comprehensive physical exam is important
- The multidisciplinary FASD Diagnostic Assessment is a "road map" to individualized recommendations and family support; it is important that individuals diagnosed with FASD and their families understand their assessment and diagnosis using strength-based language and strength focused recommendations.

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Introduction

Fetal Alcohol Spectrum Disorder is characterized by patterns of neurobehavioural impairments and physical characteristics associated with prenatal alcohol exposure. It is now recognized as a prevalent disorder, with recent North American studies citing the occurrence of up to 5.0% in some American communities and a global prevalence suggested in a recent systematic review of 1% [1]. Significant variability in prevalence is also noted with much higher prevalence in high-risk populations such as those in foster care or in mental health

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studies [1, 2]. Alcohol use in pregnancy is also common with estimates of 10–15% of women using alcohol during pregnancy [3]; it is important to recognize that many women stop or reduce their alcohol consumption when they realize they are pregnant [4].

Early diagnosis has been long recognized as a protective factor in the care of children who have been prenatally exposed to alcohol [5]. Early diagnosis of the child facilitates early intervention, reframes expectations, avoids inappropriate labeling, supports areas of challenge, encourages the development of areas of strength and resilience, and allows for access to appropriate and specific resources. It provides direction and guidance for interventions, a caregiver "map", and creates the opportunity for a better fit between the child and their environment. It also increases the ability of the physician to advocate for appropriate services for the child as well as education and support for their parents. Finally, it informs the development of community-based networks of programs and policy development that both support the individual and family in their community, as well as the development of prevention programs at all levels.

At present, there is no single clinical diagnostic test for FASD. Therefore, guideline recommendations focus on multidisciplinary assessments that describe the comprehensive medical, neuropsychological, affective, and adaptive functioning of an individual who has been exposed prenatally to alcohol in significant amounts that are recognized to be associated with global effects on functioning [6]. It is also important to consider medical and genetic differential diagnoses and the impact of environmental stressors. There are a number of genetic and teratogenic conditions with similar facial dysmorphology [7]. The neurobehavioural effects may be confounded by significant environmental stressors both prenatally and postnatally, adverse prenatal nutrition, and effects of complex familial learning disorders. Research is increasingly recognizing the epigenetic influences of environmental stresses such as poverty, abuse/neglect or emotional deprivation, on gene expression [8].

The clinical assessment of children and adults who have been prenatally alcohol-exposed is defined by various guidelines established in Canada, the United States, and Australia [6, 9, 10]. While there has been considerable discussion regarding differences among the guidelines, there are shared principles which should be considered by the diagnostician and their community:

- Developmental screening and screening for prenatal substance use
- Confirmation of prenatal alcohol exposure (PAE)
- Comprehensive physical and neurobehavioural assessments
- Differential diagnosis
- Confirmation of diagnosis and comorbidity
- Follow-up and longitudinal care

Confirmation of Prenatal Alcohol Use

Confirmation of prenatal alcohol use can be a challenging area for many clinicians. They may not feel comfortable eliciting the information if they have a relationship with the biological mother, and they may feel conflicted about their roles in managing the information once it is shared. It has been commonly stated that "FASD is 100% preventable", however in practice, this has not been realized. Many pregnancies are not planned and normative social drinking can easily surpass the binge threshold for women as defined in the guidelines of 4 drinks on a single occasion [6]). In addition, several weeks or months of pregnancy frequently pass before a woman recognizes she is pregnant, potentially further exposing the fetus to alcohol if there is continued use.

Before talking with a birth mother it is helpful to have some knowledge about why women use alcohol during pregnancy. It is critical to convey to a birth mother that she is not being judged or blamed, and that support is available. Some of the reasons women may use alcohol in pregnancy include

- not recognizing they are pregnant
- lack of information on the effects of alcohol on a fetus
- addictions
- intimate partner violence/pressure
- peer pressure
- blocking painful memories of adult or childhood trauma
- coping with stress.

When talking with a birth mother about alcohol, it is important to invite her to engage in a balanced and non-judgmental discussion about her concerns. It is equally important to respect a birth mother's decision NOT to agree to a discussion or to not disclose alcohol use; an educational approach which provides information on why some women use alcohol, along with information on supports available offers her information on harm reduction options and leaves the door open to follow-up discussion. It is important to assess her reaction to the discussion and provide support as needed.

It is helpful to be aware of some common feelings birth parents have such as shame, feelings of guilt, fear, or anger at themselves. It can be helpful to place their alcohol use in the context of societal use; it may be helpful to emphasize that alcohol use in pregnancy is complicated and often relates to broader societal contexts for which she is not responsible (eg. intergenerational trauma). It is important to reassure the woman that you will not be using information about prenatal alcohol use "against her" (ie. to prevent reunification or to apprehend children she may be parenting). Avoid using words that induce guilt/shame/or blame (ie. "If you don't give us this information your child won't get the help they need"). Disclosure of alcohol information can be a process that occurs over time—leave the door open for her to talk more with you at a subsequent appointment. The benefits of

a supportive and open discussion with a birth mother is that she can provide direct information about alcohol use and it enables her to potentially be part of the assessment process; if her child is diagnosed, her involvement with the child will benefit from her having an understanding of the disability.

It is recommended that children over age 12 who are cognitively able to understand they are undergoing assessment be aware that they are coming for an FASD assessment. In this context, preserving the relationship between the birth mother and the child is very important even if the child isn't living with their mother. It is also important when talking with a child or youth to use supportive language and consider terms such as "brain differences" or "learning differences" rather than phrases such as "brain damage". Younger children may not be developmentally ready to be told ahead of time that they are having an FASD assessment, however, it is important to plan for sharing the diagnosis with them in a supportive and developmentally appropriate way at a later time to promote self-understanding.

There are a number of benefits to comprehensive assessment and diagnosis. The diagnostic assessment can promote understanding of an individual's needs and facilitate the provision of appropriate services across the lifespan. The assessment informs appropriate adaptations to the environment around the individual and builds a circle of support with respect and understanding. Recommendations should provide direction and guidance for interventions thus creating a better fit between child and environment:

- Increases ability to advocate for appropriate services.
- The appropriate diagnostic terminology redirects consideration of inappropriate labelling of behaviour and supports a focus on the strengths of the individual.
- The diagnosis provides an opportunity for the individual and their family to explain and receive greater understanding and support from extended family and friends as well as those in their community.
- Children can learn about their own learning styles and needs and, as they grow, older children and youth can learn to become their own advocates in their community.

Symptoms of FASD across the Lifespan

Symptoms related to prenatal alcohol exposure develop as an individual ages, depending on the neurological maturity, health, environmental influences, and individual resiliency. It has been suggested that one of the more significant effects of PAE is relative dysmaturity of various neurological domains of functioning. Commonly, the combination of executive dysfunction, cognitive dysfunction, and neurologic sensitivity often results in adaptive dysfunction to a greater degree than might be expected given the measured cognitive potential. The summative impact of these challenges directly related to PAE often results in significant difficulty in completing complex tasks in all aspects of functioning and in the individual's ability to understand and predict the consequences of actions. Without an appropriately adapted environment and supportive family, the individual frequently struggles to function successfully in daily activities.

It is important to recognize that the outcomes of PAE vary considerably dependent on the complex interactions of gestational timing of exposure, dose and frequency, maternal genetic metabolism of alcohol, and environmental factors including maternal nutrition and other stressors [11]. While the psychiatric comorbidities associated with FASD have been previously described as "secondary disabilities" related to the stresses of not being appropriately supported or understood, new research on the disruptive effects of PAE on the developing hypothalamic-pituitary axis and various neurotransmitter systems suggests that these comorbidities are a direct and primary result of exposure [11]. New research on the "epigenetic signature" of FASD, or unique DNA methylation patterns related to PAE, is seeking to provide a biomarker for FASD which could identify individuals needing early intervention and further reduce comorbidity [12].

Common Symptoms in Infancy

Infants born with a history of significant prenatal alcohol exposure frequently experience difficulties with self-regulation in ways that are observable in the nursery. Some of these infants may demonstrate neonatal abstinence syndrome (NAS), however, the presence of NAS is a function of the timing of prenatal alcohol exposure and so the absence of NAS does not mean the absence of exposure. Infants who have had greater exposure early in the pregnancy may not demonstrate NAS but may still struggle with state control.

Common symptoms in infancy include:

- Excessive arousal
- Sleep problems
- Short attention
- Developmental delay
- Motor abnormalities-delay, abnormal quality
- Abnormalities in tone, reflexes
- Atypical sensory responsivity

Common Symptoms in Preschoolers

As children enter preschool, the developmental tasks of the preschooler begin to be challenged by the difficulties with self-organization and self-regulation seen in children with prenatal alcohol exposure. Some behaviours such as aggression may reflect difficulties with attention regulation, developmental impulsivity, and past adverse life experiences.

Common symptoms in preschoolers include:

- Hyperactivity
- Attention problems
- Language delay
- Motor incoordination
- Atypical sensory responsivity (e.g. delayed auditory processing)
- Aggression
- Abnormal memory
- Delayed play skills

Common Symptoms in School Age

The school-age child with FASD exhibits typical dysfunction in a number of domains of learning, self-regulation, and social development. Typically, many of these children demonstrate impairments in the regulation of attention as well as learning difficulties. They may also struggle with a social naiveté or immaturity that may result in them following others into difficult or inappropriate behaviours.

Common symptoms in school-age children include:

- Working memory deficits
- Severe language impairment
- Sensory issues are often overlooked
- Poor judgment
- Learning disability, weak academic achievement/academic failure
- Unstable social situations
- Psychiatric co-morbidity
- External support needed for success

Common Symptoms in Adolescents

In addition to the difficulties experienced by the school-aged child with FASD, the adolescent is developmentally expected to take on early adult roles and prepare themselves for the independent functioning of adults in school or in occupations. The adolescent with FASD however, may be challenged with making appropriate judgements in social situations and can be highly at risk for victimization or exploitation. Their gaps in abstract reasoning and forming associations can lead to difficulties predicting the consequences of behaviour, and impulsivity can lead them to inappropriate behaviours.

Common symptoms in adolescents include:

- Working memory deficits
- Severe language impairment
- Sensory issues are often overlooked
- Gaps in thinking process—difficulty forming associations, predicting, abstract reasoning, cause and effect, generalization
- Learning disability
- Impulsivity and distractibility
- Difficulty managing free time
- Difficulty with judgments ie. weighing and evaluating—difficulty understanding safety and danger—heightened risk of exploitation and victimization
- Difficulty managing time, money, and schedules
- Social dysmaturity
- External support needed for success

Adults with FASD

Adults with FASD are frequently unrecognized and, without appropriate coaching and community supports across systems, may struggle with independent functioning. There is increasing recognition of FASD among those who struggle with other mental health issues including anxiety and depression. Their multiple challenges may result in homelessness, unemployment, substance use, and difficulties with the law, and these difficulties may be persistent without appropriate support. There is a significant need for appropriate vocational training and an informed circle of support around the adult with FASD.

Diagnostic Process and Multidisciplinary Assessments

The Canadian diagnostic guidelines describing the FASD diagnostic process recommend a multidisciplinary approach to assessment which may occur over time [6]. The guidelines recommend a team comprising a physician with training in FASD dysmorphology, psychology, speech and language therapy, and occupational therapy; additional recommended team members may include social work, behavioural support/follow-up, or nursing. A comprehensive assessment includes an evaluation of the individual's physical features, medical status, and brain domains of functioning; confirmation of alcohol exposure is required in amounts described in guidelines consistent with adverse effects on development. It must also carefully consider both prenatal and postnatal influences on physical, emotional, and cognitive development, including the effects of trauma, attachment, and toxic stressors.

Typically each team member contributes to the comprehensive neurobehavioral assessment: the speech-language pathologist is responsible for the language assessment; the

occupational therapist contributes to the motor, visual motor integration, and sensory processing; the psychologist assesses cognition, academic achievement, memory, executive function, attention, adaptive functioning, and affect regulation.

The roles of the physician include confirmation of alcohol use, comprehensive history taking, complete physical examination including dysmorphology, differential diagnosis, and diagnostic formulation. The physician leads diagnostic team discussions both before and after all the assessments are completed in order to integrate information into a comprehensive diagnostic formulation. This formulation considers all the appropriate diagnostic determinations and factors that may influence severity. For example, an individual may be diagnosed with FASD, Intellectual Disability, and Anxiety Disorder. It is incumbent on the physician to work effectively within the multidisciplinary team to ensure that a thorough and comprehensive assessment as recommended in the guidelines is completed in order to properly inform diagnosis. Multidisciplinary training is available online through the CanFASD website (https://canfasd.ca/online-learners/) or through the Washington Diagnostic and Prevention Network (https://depts.washington.edu/fasdpn/htmls/team-train.htm).

Medical Assessment

There is an increasing body of literature linking the effects of prenatal alcohol exposure on various body systems. According to a recent meta-analysis, the most frequent systems impacted include the special senses (including hearing) and peripheral nervous system, receptive and expressive language, and psychiatric/neurologic systems, with the most prevalent conditions within congenital malformations and mental and behavioural disorders [13]. It is thus important to ensure that the medical assessment is thorough and complete to both ensure that all systems are fully examined, and to inform a complete differential diagnosis.

Differential diagnosis of the prenatally alcohol-exposed individual is critically important. Information from the multidisciplinary team should inform this differential as medical, genetic, and postnatal environmental factors need to be considered carefully. Environmental stresses as well as positive and supportive environments can modify the expression of neurobehavioural outcomes, increasingly recognized to be moderated through epigenetic influences. Thus, a diagnostic approach that considers the multiple features impacting neurodevelopment and behaviour including trauma/intergenerational trauma, the impact of disrupted attachment, and other toxic stressors is important. Medical conditions that have been frequently related to prenatal alcohol exposure include prematurity, congenital cardiac malformations, congenital neurologic malformations (such as neuronal migration abnormalities, other structural brain malformations), seizure disorders, and orthopedic malformations, also occur without prenatal alcohol exposure. Finally, a number of other genetic and other conditions associated with teratogenic exposures can mimic some of the facial dysmorphology; these conditions may include primary genetic disorders such as DiGeorge Syndrome or Ritscher-Schinzel Syndrome, or disorders related to other prenatal exposures such as Fetal Hydantoin or Fetal Valproate Syndromes. [7].

Assessment of FASD Dysmorphology

The sentinel facial features in FASD described in the Canadian Guidelines, and consistent with the Washington Diagnostic Prevention Network four-digit code, are short palpebral fissures, smooth formation of the philtrum, and a thin volume of the upper lip, clinically significant at less than 2 standard deviations from published population means (Fig. 22.1) [6, 14].

Measurement of the eyes, lip and philtrum should be undertaken by a clinician trained in measurements. Training is available through the Washington Diagnostic and Prevention Network (http://depts.washington.edu/fasdpn/htmls/training.htm). The palpebral fissures are measured from the endocanthion (where the inner eyelids meet) to the exocanthion (where the lateral eyelids meet) for each eye; palpebral fissure lengths shorter than the third percentile based on appropriate norm references are clinically significant (Figs. 22.2, 22.3, and 22.4) [15]. The

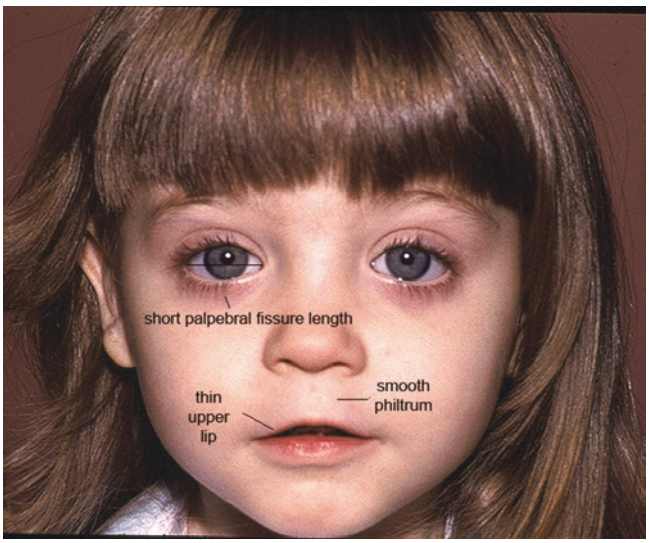

Fig. 22.1 "Child presenting with the three diagnostic facial features of FAS: (1) short palpebral fissure lengths (distance from A to B); (2) smooth philtrum; and 3) thin upper lip. Copyright 2019, Susan Astley PhD, University of Washington."

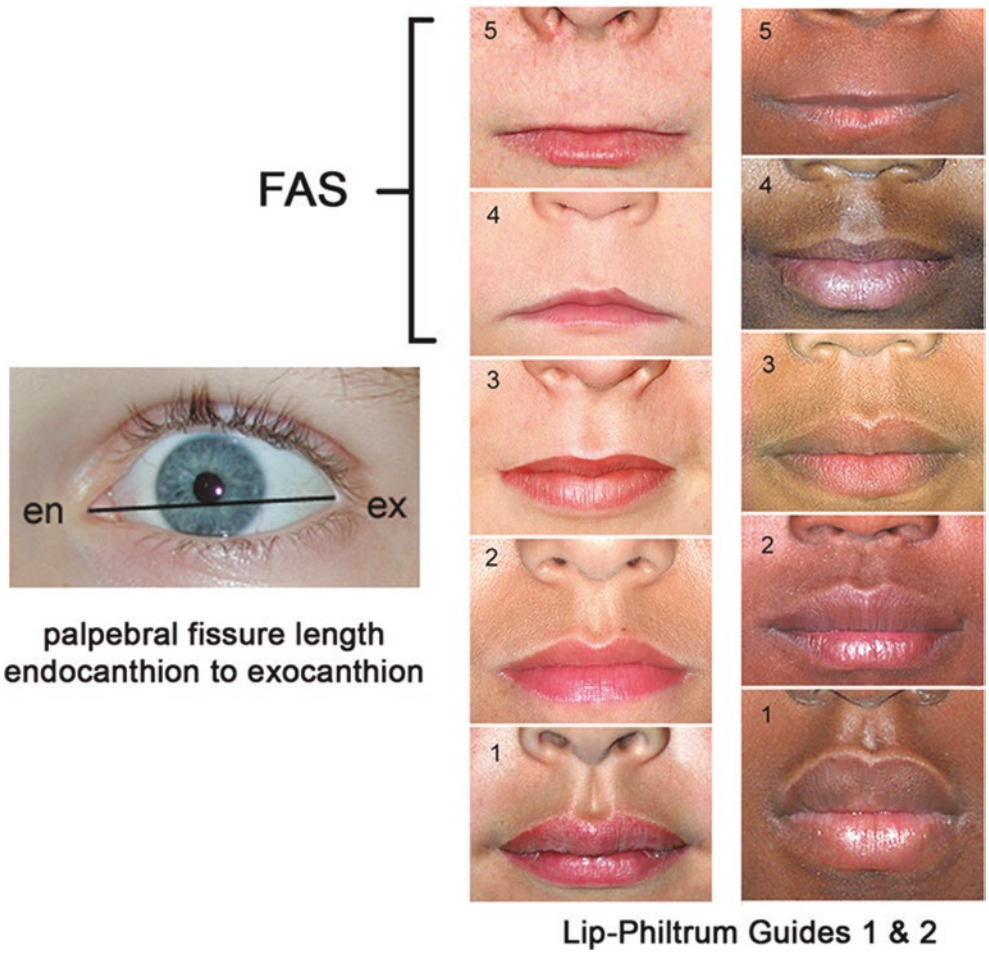

Fig. 22.2 Measuring the eyes, lip, and philtrum in FASD: "The three diagnostic facial features of FAS include: (1) short palpebral fissure lengths, (2) A smooth philtrum (Rank 4 or 5 on the Lip-Philtrum Guide), and (3) a thin upper lip (Rank 4 or 5 on the Lip-Philtrum Guide). Lip-Philtrum Guides 1 and 2 are used to rank upper lip thinness and philtrum smoothness. The philtrum is the vertical groove between the nose and upper lip. The guides reflect the full range of lip and philtrum shapes with Rank 3 representing the population mean. Ranks 4 and 5 reflect the thin lip and smooth philtrum that characterize the FAS facial phenotype. Guide 1 is used for Caucasians and all other races with lips like Caucasians. Guide 2 is used for African Americans and all other races with lips as full as African Americans. Copyright 2019, Susan Astley PhD, University of Washington."

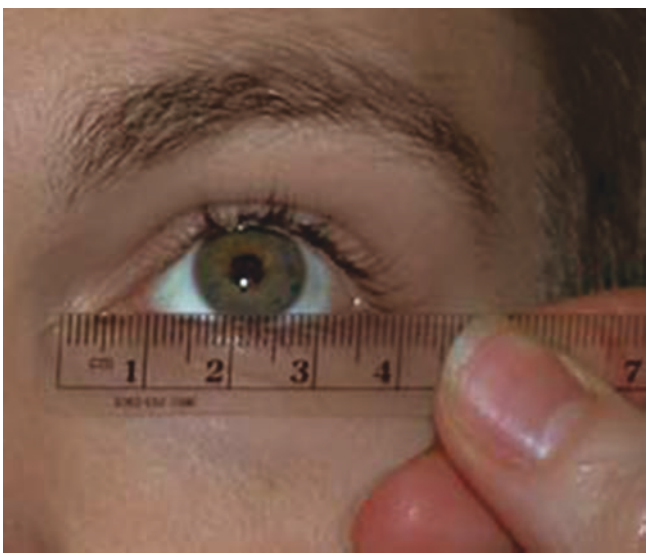

Fig. 22.3 "The palpebral fissure length (the distance from the inner corner to outer corner of the eye) being measured with a small plastic ruler. Copyright 2019, Susan Astley PhD, University of Washington."

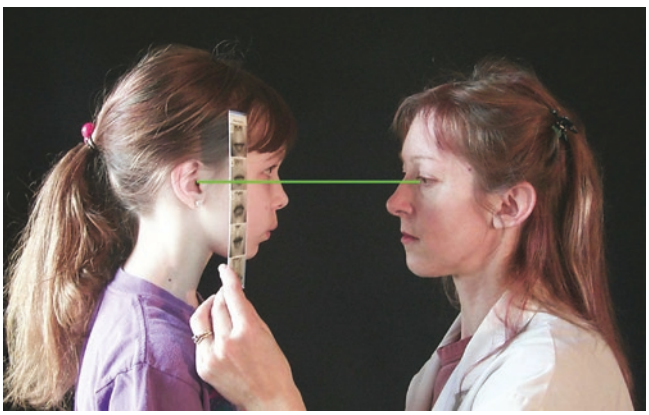

Fig. 22.4 "Physician using Lip-Philtrum Guide. Illustration of a physician aligned in the patient's frankfort horizontal plane while using the Lip-Philtrum Guide to rank upper lip thinness and philtrum smoothness. The frankfort horizontal plane is defined by a line (green line) that passes through the patient's external auditory canal and the lowest border of the bony orbital rim (orbitale). The physician's eyes (or camera lens) should be directly in line with this plane. If the physician stood above this plane looking down on the patient, the patient's upper lip could appear thinner than it truly is. Click on this weblink (http://depts.washington.edu/fasdpn/htmls/fas-tutor.htm#frankfort)to see an animation demonstrating how to align yourself in the patient's frankfort horizontal plane. Copyright 2019, Susan Astley PhD, University of Washington"

volume of the upper lip or vermillion border and the smoothness of the philtrum should be compared against the Lip-Philtrum Guide published by the University of Washington Diagnostic Prevention Network (Figs. 22.5 and 22.6) (http://depts.washington.edu/fasdpn/htmls/fas-face.htm).

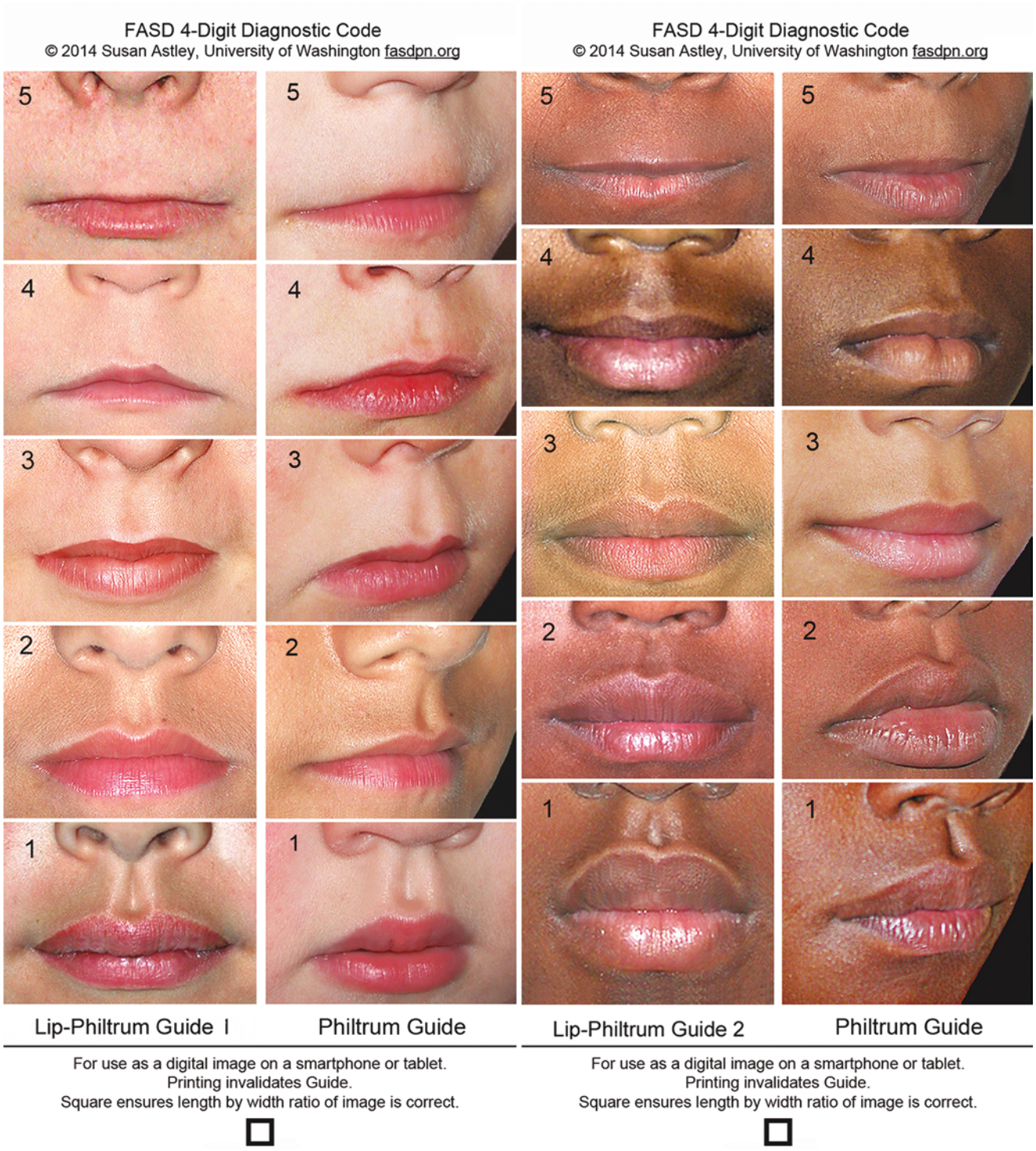

Fig. 22.5 "University of Washington Lip-Philtrum Guides 1 (**b**) and 2 (**b**) are used to rank upper lip thinness and philtrum smoothness. The philtrum is the vertical groove between the nose and upper lip. The guides reflect the full range of lip thickness and philtrum depth with Rank 3 representing the population mean. Ranks 4 and 5 reflect the thin lip and smooth philtrum that characterize the FAS facial phenotype. Guide 1 is used for Caucasians and all other races with lips like Caucasians. Guide 2 is used for African Americans and all other races with lips as full as African Americans. Free digital images of these guides for use on smartphones and tablets can be obtained from astley@uw.edu. Copyright 2019, Susan Astley PhD, University of Washington"

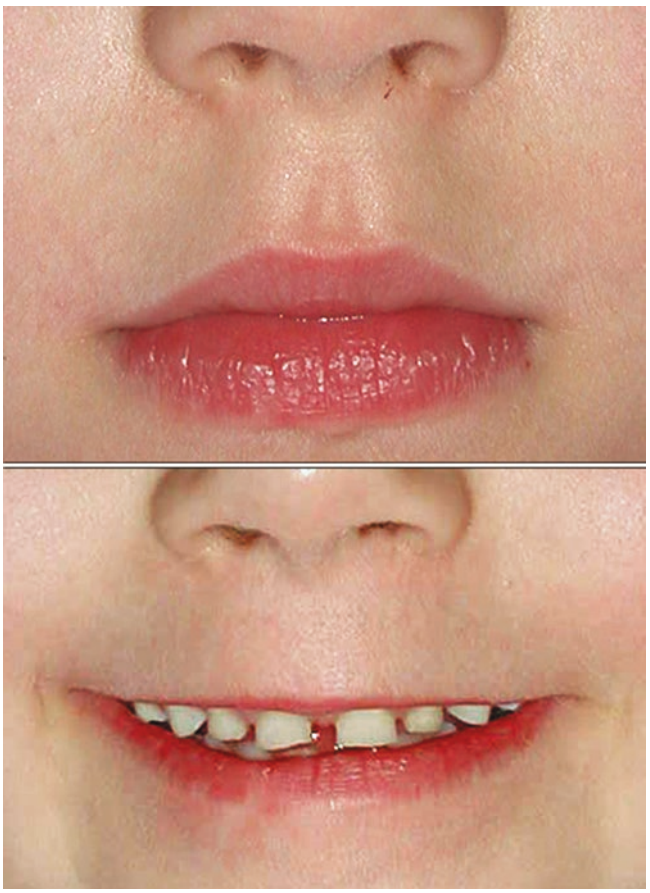

Fig. 22.6 "Lips must be gently closed with no smile to accurately measure philtrum smoothness and upper lip thinness. This is the same child with and without a smile. A smile will make the philtrum appear smoother and the upper lip to appear thinner than they truly are. Note that without a smile, the lip and philtrum would both receive a correct Likert Rank of #2 on the Caucasian Lip-Philtrum Guide. With a smile, the lip and philtrum would both receive an *incorrect* Likert Rank of #5. Copyright 2019, Susan Astley PhD, University of Washington."

There are a number of other features which have been described in association with FASD but which are non-specific, and therefore cannot be relied upon for diagnosis. However, the features should be recorded carefully and consultation with genetic specialists should be accessed as needed for further testing to inform differential diagnosis. These features may include:

- Railroad track ears,
- Mid face hypoplasia
- Micrognathia
- Ear abnormalities
- High arched palate
- Epicanthic folds
- Limb abnormalities
- Abnormal palmar creases
  Short up-turned nose

Multidisciplinary Neurobehavioral Assessment

Despite a considerable body of research, there is no single neuropsychological profile that is specific to FASD. Most guidelines require evidence of pervasive or global brain dysfunction described by neuropsychological measures in specific domains at or below 2 standard deviations from the mean, in at least 3 or more different domains [6, 9, 10]. These functional measures may be both direct observational measures as well as reported measures from reliable informants such as parents, caregivers, teachers, and community support workers. They should reflect the individual's typical level of functioning.

The Canadian Guidelines [6] describe the following domains of brain function that need to be assessed:

- Motor Skills
- Neuroanatomy/neurophysiology
- Cognition
- Communication
- Academic achievement
- Memory
- Attention
- Executive Function
- Affect Regulation
- Adaptive behaviour, social skills, or social communication

The DSM-5 describes Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE) in their appendix of conditions for further study [16]. It conceptualizes the domains of functioning into three groups: neurocognitive functioning (full-scale IQ less than or equal to 70 on a standardized IQ test, executive functioning, learning/academic achievement, memory, and visual-spatial reasoning), self-regulation (including mood or behavioral regulation impairment, and attention deficit), and adaptive functioning (including deficits in at least one domain of communication or social impairment, and impairments in daily living and/or motor skills) [16].

It is important to take a developmental or life course approach and consider these domains by age, as some may be more observable or measurable at different life stages. The following table describes brain domains to be considered by age. The domains listed in Table 22.1 consider those recommended by the Canadian Guidelines, as well as the previously described domain of sensory processing for additional consideration by age. The table below describes a relative clinical and developmental weighting of various domains to each age group.

Table 22.1 Clinical domains in FASD assessable by age group (+ = easily accessible using standardized measures; – = not assessable using standardized measures)

Domains of Function				
	Birth—infancy	Preschool	School age	Adolescence/adult
Neuroanatomy/neurophysiology	++++	++++	++	++
(sensory processing)	++++	+++	+	+
Motor skills	++++	++++	++	++
Cognition	+	++	++++	++++
Academic achievement	–	++	++++	+++
Communication	+	++++	+++	++
Memory	–	+	+++	++++
Attention	+	++	++++	+++
Executive function	–	++	+++	++++
Affect regulation	+	+	++	++++
Adaptive behaviour	–	+	+++	++++

Case Example of FASD Diagnostic Assessment by Age

The following case is compiled from clinical situations seen frequently and does not represent a specific case history. The information is intended to illustrate several "clinical pearls" which demonstrate key assessment points for the clinician in a diagnostic team. The case information is presented in italics; the clinical pearls are discussed below. Elements of the case representing preschool information, school age, and adolescent, age groups are provided to also highlight some of the key clinical considerations in each of these age groups.

Case Example: April

Referral History

April is a 7-year-old girl who lives with her long-term foster parents. She was taken into care as an infant shortly after birth. She was referred for assessment of possible FASD by her social worker who was concerned about her prenatal alcohol exposure as well as her foster parents' concerns about her overall development.

Clinical Pearls:

Referral criteria should be established by the clinic and handled through an intake process.

We recommend that the following be considered:

1. Prenatal Alcohol Exposure (PAE) Confirmation—*We recommend reliable confirmation of prenatal alcohol exposure be established prior to assessment*. When it is not feasible or possible to obtain confirmation of PAE directly from the birth mother, reliable collateral information can be used. Sources of reliable collateral information can include medical records, social services agency documentation, and direct observation of family members. Caution should be exercised where collateral information is based on suspicion, biased, or third-hand. Sometimes it is not possible to obtain reliable confirmation of PAE; t is recommended that in the absence of classical dysmorphology and microcephaly, a diagnostic assessment for FASD not proceed. Typically, this may occur in instances of foreign adoption, where a diagnosis of the neurodevelopmental disorder may be supportable on clinical evidence and with appropriate investigations, without specifying etiology.
2. Other criteria for assessment may include concerns of developmental, learning, or behavioural difficulties. *Assessment of individuals with prenatal alcohol exposure without evidence of developmental or behavioural difficulties does not necessarily result in meeting diagnostic criteria*. Timing of the assessment should be carefully considered, including not proceeding with the assessment.
3. *Consent for assessment must always be clear, informed, and present.* Situations where consenting to the assessment may be complicated may include situations where the parent/child dyad is stressed or fragile, adolescents who may be struggling with their relationship with the biological parent, individuals with evolving mental health difficulties, and individuals with ambivalent or traumatic parent relationships. These situations do not necessarily preclude assessment but should be carefully undertaken with a professional assessment of the timing and support needed.

Social Background

April lives with her foster parents and their two adult biological daughters. Her foster mother stays at home to look

after April. Her foster father works as a school teacher. April has lived with this family since she was five days of age. She does not have any contact with her biological family and is a permanent ward of a child and family services agency. Reports indicate that the birth mother lives a transient lifestyle with chronic alcoholism. She has tried alcohol treatment a number of times in the past but has not been able to complete treatment programs.

Clinical Pearls:

Research has shown that there is a high percentage of children in care who have family histories of alcohol and substance use and mental illness [17, 18].

Even when a child is in permanent care, it is important to be respectful and supportive of the relationship or memory the child may have with their biological parent.

It is also important to recognize that histories of chronic alcoholism or non-completion of treatment programs do not in and of themselves confirm PAE. Individuals who experience chronic alcoholism may also have periods of abstinence which should be specifically elicited and recognized, such as time in treatment even if not completed, voluntary abstinence, or involuntary abstinence (eg. hospitalization or incarceration). To meet the definition of alcohol exposure required by the Canadian Guidelines, PAE must occur during the pregnancy in question in significant amounts defined as 7 or more standard drinks per week or two binges (4 or more drinks on the same occasion) [6].

Prenatal Alcohol/Drug Exposure History

April's birth mother confirmed alcohol use in a binge pattern throughout all three trimesters of the pregnancy. The use of crack cocaine, tobacco, and marijuana by the birth mother during pregnancy was also confirmed.

Clinical Pearls:

Histories of multiple substance exposure are common.

The Canadian Guidelines recommend that "all pregnant and postpartum women should be screened for alcohol use with validated measurement tools" and that "Women at risk of heavy alcohol use should receive early, brief interventions" [6].

There are a number of toxicology tests designed to identify prenatal substance use using various matrices including neonatal meconium, umbilical cord blood, and urine analysis, as well as maternal urine toxicology, to name a few. Each of these tests has different indications and sensitivities, reflect different timings of exposures, have different geographical availabilities, and used in combination may provide inconsistent results (Fig. 22.7) [19].

These tests, if used, should be interpreted by experienced and informed clinicians. False positives (for example, related to cross-reactivity of the antibody to other substances such as prescription medications or dietary metabolites) and false negatives (for example, related to low concentrations or insufficient sampling) may have significant medico-legal and social implications [20].

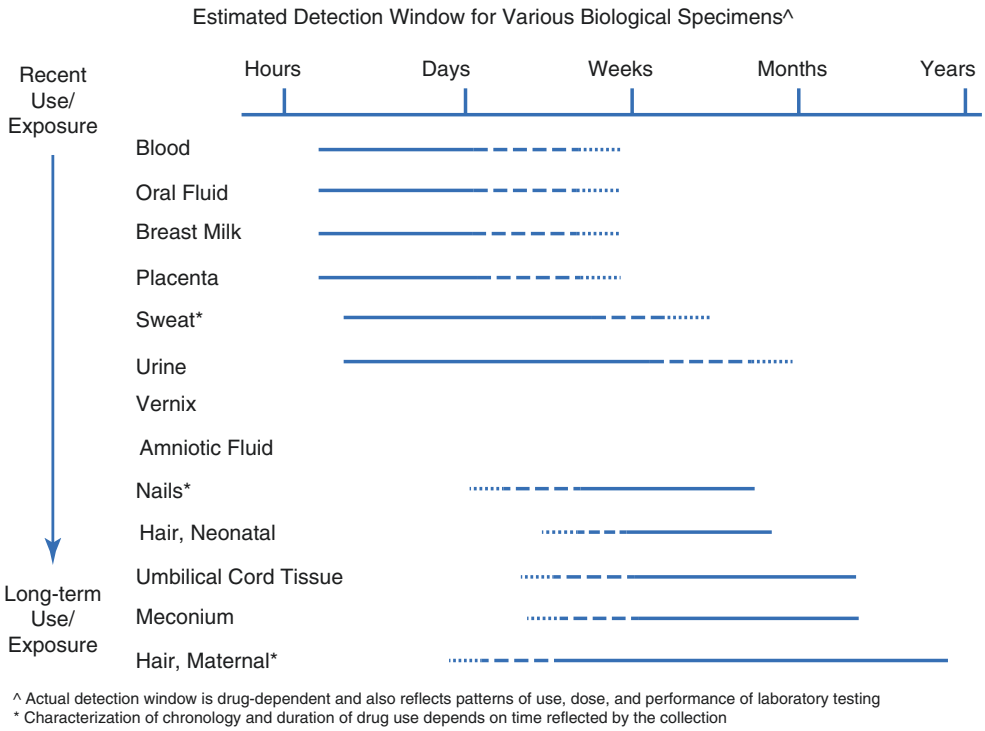

Fig. 22.7 Estimated detection window for various biological specimens (taken from: [20])

Maternal/Neonatal History

April was born to a 25-year-old G9P7 woman at 36+ weeks gestation by spontaneous vaginal delivery.

Apgar scores were 8 at 1 minute and 9 at 5 minutes. Birth weight was 2782 grams (25th%ile), length was 47 cm (10-25th%ile). Head circumference was 32 cm (10-25th%ile).

Clinical Pearls:

Growth remains a key component of a comprehensive pediatric physical examination, but somatic growth is no longer a distinguishing feature of FASD in the Canadian Guidelines due to its lack of specificity [6]. However, there is research which supports the continued consideration of growth restriction in the diagnosis of FASD [21]. Growth continues to be a key feature in the American nomenclature including the Washington 4-digit code, the Hoyme guidelines (where the thresholds for growth and head circumference are at or below the tenth percentiles) and in other international guidelines [9, 22]. In addition, abnormalities in head circumference, particularly *microcephaly defined as head circumference less than 2 standard deviations below the mean, is considered part of the domain of "neuroanatomy/neurophysiology"* along with other features such as abnormalities on imaging or electroencephalogram (EEG) [6].

There were no neonatal complications noted. Prenatal alcohol exposure (PAE) was denied at birth and was not recorded on the medical chart. Symptoms of neonatal abstinence were not recorded.

Clinical Pearls:

It is common for the medical chart to not have a history of PAE and it may have conflicting or suspected information on alcohol and other substances used. The prenatal and delivery periods are vulnerable periods for a woman who may be concerned about her use of alcohol but who is also concerned about potentially having her child removed from her care. Alcohol and substance use histories should be screened for and elicited in the prenatal period by trusted, trauma-informed clinicians who can work with a woman to provide harm reduction counselling and substance use treatment.

Medical and Family History

April has had repeated ear infections with suppuration. T-tubes were placed when she was 3 years old and no further problems with ear infections or hearing were reported. Her review of systems is unremarkable; there is no history of seizures, chronic illnesses, hospitalizations or head injuries.

There are no known genetic disorders in other family members. It is not known whether other children have been diagnosed with FASD in the family.

Clinical Pearls:

FASD is increasingly being described as a "whole body disorder" with teratogenic impacts on multiple body systems, especially cardiac, gastrointestinal, and endocrine systems, but with documented co-morbidity across all major systems [13, 23]. It is recommended that a comprehensive history and physical exam be regularly completed by the individual's primary care physician with regular appointments for health monitoring and anticipatory guidance provided by the primary care practitioner [24].

Developmental History

April's foster mother reports that her speech and language were delayed but her motor developmental milestones were relatively normal. She has had concerns regarding self-regulation and high activity since she was a toddler and she reports that April still doesn't engage with any toy or activity for longer than a few minutes. She can get very upset at times over small triggers and cries easily. She enjoys playing with other children, although she can get aggressive with them if she wants attention, or if she is upset by something. April is otherwise described as affectionate and caring. She can be overly friendly with strangers.

Clinical Pearls:

Some behaviours such as temper tantrums and aggression in young children may have multiple contributing factors including regulation of attention and impulsivity as well as past adverse life experiences. It is important for an experienced clinical team to carefully consider the impact of various life experiences including trauma and attachment on the behavioural assessment of a child with PAE.

In reviewing her daily activities, April is a good eater and is not picky. She always smells her food before she eats it. There is no history of choking or gagging and no other oral motor concerns. She was toilet trained later than expected and was sensitive to the sounds of flushing toilets. April has always been a restless sleeper who takes time to fall asleep. Her family provides her with a calming bedtime routine and recently has started treating her with Melatonin.

Clinical Pearls:

Sensory processing differences are very common in children with PAE and can have a significant impact on difficulties with daily functioning [25]. Children with PAE have more difficulties with sensory processing and sensory-motor activities with resulting impacts on academic achievement and adaptive behaviours impacting home and school [26]. These differences in sensory processing can impact daily functioning, feeding, and sleep in children with FASD [26–

29]. ***Early referral to occupational therapy for assessment, education, and environmental modification is recommended.***

Children with FASD may have disordered sleep related to altered melatonin secretion, circadian rhythm abnormalities, and possibly sleep-disordered breathing [27, 28, 30***].*** The relationship between disordered sleep and mood disorders is well documented, however, the effect of prenatal alcohol on the development of internalizing and externalizing behaviours into adolescence and adulthood is being increasingly recognized [31, 32].

April is now in a regular grade 2 program. She enjoyed kindergarten and has always been described as outgoing and friendly. The school has involved their speech and language therapist as April continues to have difficulty understanding and tends to speak in short sentences. She sometimes misses social cues but overall, she was described as a very social young girl. Concerns were expressed about early literacy, numeracy, fine motor skills, and memory. Challenges identified by the schools included her short attention span, task completion and retention of information. She has been referred to the school resource program.

Clinical Pearls:

Children with PAE are at risk for receptive and expressive language impairments as well as speech disorders and should be screened early and referred to speech and language pathology for early intervention and regular monitoring [33, 34***].*** It has been suggested that there is a neuromotor component to the speech impairment and assessment of oral motor planning and auditory discrimination should be part of the comprehensive assessment [33]. ***Social pragmatic language and behaviour should also be assessed as difficulties may be independent of cognitive functioning and impact daily adaptive behaviours [***35, 36***].***

Evidence-based programs supporting early math interventions, attention and self-regulation, and academic support are helpful for children with FASD [37–39***].***

Physical Assessment

On examination, April presented as a generally healthy young girl. Her height was at the 25th%ile, her weight at the 15th%ile, and her head circumference was at the 25th percentile.

Palpebral fissure lengths were small, less than the third percentile for her age.

Her philtrum scored 4 and her upper lip scored 3 on the Lip/Philtrum Pictorial Guide.

Her palate was intact. Her ears were normally placed and formed. Her chest was clear and heart sounds were normal. Upper extremities revealed normal palmar creases, normal joint mobility and normal nail formation. Neurologic exam was normal.

Clinical Pearls:

Most individuals with PAE do not show full dysmorphology and are often overlooked for comprehensive assessment. Careful history taking and screening for PAE can support comprehensive assessment for FASD. April's physical exam shows only one of the 3 sentinel facial features. She would not meet the facial dysmorphology criteria using the Canadian Guidelines [6].

Neurodevelopmental Assessments

April was quite active and needed to be frequently redirected to the task at hand. However, she cooperated to a certain extent with most of what was presented to her.

Psychological Assessment

Cognitive

- April's overall cognitive performance was found to be in the Below Average range (FSIQ = 75, fifth percentile), with clinically significant variability among domains and individual skill areas.
- Moderate impairments (second to eighth percentile, Very Low range) were noted with respect to other elements of cognitive functioning (overall cognitive proficiency, quantitative reasoning, nonverbal skills), academics (alphabet fluency, math)

Adaptive/Social

- Parent and teacher ratings of April's adaptive skills were in an impaired range (GAC = 51 and 54, respectively, both <first percentile, Extremely Low range).
- Parent and teacher ratings also flagged at-risk concerns regarding atypical behaviours and attention
- April's performance was broadly intact (Low Average range) on comprehension of social conventions.

Executive Function

- Areas of executive functioning (lexical fluency, concept formation, rule-following) were scored in the Low Average range.
- Moderate impairments (second to eighth percentile, Very Low range) were noted with certain elements of attention and executive functioning, including selective attention.

Memory/Attention

- April scored in the Low Average range (ninth to 24th percentile) on overall visual fluid reasoning, visual memory, and sustained attention
- Moderate impairments in elements of rote verbal memory and spatial memory
- Personal strengths in visual-spatial construction, such as visual working memory, visual scanning, and visual-spatial planning (Average range, 25th to 75th percentile) were noted.

Communication/Language

- Significant impairments (<first to first percentile, Extremely Low range) emerged on tests of overall verbal reasoning and overall verbal working memory
- Low average range in expressive vocabulary
- Overall, April's verbal skills were weaker than her visual skills, though across both areas, there were large discrepancies in performance.

Occupational Therapy Assessment

Shore Handwriting Screening for Early Handwriting Development.

- Fine motor skills appeared to be delayed compared to skills of children at her age level.

The Beery Developmental Test of Visual-Motor Integration (VMI)

- April scored in the Below Average range while the Visual Perception and Motor Coordination subtests were Average and Very Low respectively.

Movement Assessment Battery for Children (MABC-2)

- Results indicated adequate motor skills with the Manual Dexterity component in the fifth percentile.
- The Aiming and Catching component was in the 37th percentile.
- The Balance component was in the 63rd percentile.

The Short Sensory Profile Caregiver Questionnaire

- Completed by her parents: the questionnaire indicated Definite Difference scores in the Movement Sensitivity, Under-responsive/Seeks Sensation and Auditory Filtering sections.
- April also scored in the Probable Difference range for the Tactile Sensitivity, Low Energy/Weak and Visual/Auditory Sensitivity sections.

Speech Language Assessment

Clinical Evaluation of Language Fundamentals—fifth Ed.

- April presented herself as a pleasant and cooperative girl with a short attention span for structured activities.
- April's language testing using the CELF-5 receptive and expressive language skills was severely delayed. It was noted that she understood simple routines and could participate in simple social exchanges, but had a hard time reading social cues.
- Her speech was at times difficult to understand and she demonstrated numerous speech sound production errors that were not developmentally appropriate.

Clinical Pearls:

Clinical experience and research support the impact of PAE on multiple neurodevelopmental areas including cognitive functioning, communication, executive functioning, and motor functioning as well as the presence of neurologic findings including so-called "soft neurologic signs" and sensory processing differences [24, 29, 32, 40–43].

Final Diagnoses

FASD Diagnosis *FASD without all Sentinel Facial Features*.

Growth (prenatal and postnatal): *Normal.*
Facial Dysmorphology: *Small palpebral fissures, normal lip fullness and flat philtrum.*
Central Nervous System Domains: *Multiple areas of significant impairment including cognition, communication, adaptive, sensory.*
Prenatal Alcohol Exposure: *Confirmed.*

Clinical Suggested Recommendations

FASD Support and Education

1. FASD follow-up: educator, social worker, system navigator
2. Informational resource package
3. Supporting strengths
4. Family respite

Medical

1. Referrals to community disability services and community living
2. Referrals for therapy services: speech and language therapy, occupational therapy, physiotherapy
3. Other speciality referrals as indicated: In this case, Audiology. In general, consider the need for referrals by system: eg. neurology (eg. seizures), audiology and otolaryngology (eg. chronic otitis media), ophthalmology (eg. vision, strabismus), feeding specialist/gastroenterologist (eg. swallowing difficulties), cardiologist, respirologist/sleep program, orthopedics (eg. scoliosis) etc.

4. Bloodwork if indicated: consider hemoglobin, serum ferritin, and thyroid studies as indicated
5. MRI Brain if indicated (not in this case)
6. Genetic investigations if indicated: microarray, fragile X

School

1. School resource and guidance team
2. School therapy team
3. Individualized educational planning with educational and classroom adaptations
4. Follow-up cognitive assessment as needed in adolescence for transition planning to adult services
5. Strength based program planning

Adolescent Follow-Up

April was referred for follow-up as an adolescent, following concerns identified by her parents and school regarding academic underachievement, social difficulties, and possible emergent anxiety.

April is now 14 years old and in grade 9 in a new school where she was noted to have some initial difficulty adjusting to the new routines, new students, and generally the larger size of the school and classes. While the school had a transition plan in place and had developed and organized an Individualized Educational Plan, they were concerned about her mood and adjustment. Her last cognitive assessment was in grade 3 and her last formal language assessment was in grade 1. She has not had occupational therapy consultation for the last number of years.

Clinical Pearls:

Many adolescents may have been previously seen and diagnosed with FASD as children but not all may have had their diagnosis communicated to them.

Academically, they often require new cognitive and functional assessments as they are faced with new challenges and transition to adulthood.

Monitoring for mood and mental health should be ongoing through primary care with referrals to mental health supports and psychiatry as needed [24, 44].

As the school year progressed with support in place, April was reported to have developed some positive relationships with peers at school. She continues to be viewed as a "follower" in her social group and is easily led into risk-taking behaviours. To date, these have been relatively mild, but have included experimenting with small amounts of alcohol, and staying out late with her friends. Her parents talked with her and have established ***ground rules for contact using cues on her phone to help her remember to text them when she's out. Another group of friends have tried to encourage her to party with them but so far April has declined, although tempted as she wants to fit in.***

Clinical Pearls:

Adolescents with FASD can find themselves in risky situations by following peers and have a hard time recognizing risk or exercising judgement in questionable situations. Even when they are coached, they have a hard time applying previously learned information.

At school she was observed by her teacher to have difficulty staying in class, transitioning between tasks and completing her tasks. She was described as academically weak and was noted to have trouble communicating her ideas verbally. She continued to struggle with attention and impulsivity. She is now being treated effectively with a long-acting stimulant.

Clinical Pearls:

Significant communication difficulties are increasingly being recognized in adolescents with FASD. These difficulties relate to executive, cognitive and daily adaptive functioning, and is an area available for specific intervention [45]. In addition, difficulties with memory and social behaviour compound the academic and functional difficulties in an age group striving for social acceptance and independent functioning [46]. It has been shown that in both children and adolescents, assessments of overall IQ including verbal and nonverbal reasoning skills, math skills, and adaptive skills in the school setting predict an FASD diagnosis [47]. These co-morbid learning and attentional problems further exacerbate independent functioning in adolescents.

April's parents have established ground rules regarding electronics; her cell phone and computer are put away at bedtime, away from her bedroom as they found out her friends have texted her at night. Her parents have been concerned about links to chat rooms and texting with people she doesn't know but who have invited her to be "friends". Her parents have also noticed she was becoming more anxious about friends and attending school. She is easily overwhelmed when given multiple instructions or coaching regarding social skills and has trouble remembering rules day to day.

Clinical Pearls:

Mental health disorders including depression and anxiety can be common in adolescents and adults with FASD [13,

48]. ***Assessment of affect regulation as a brain domain as defined in the Canadian Guidelines can be challenging as it can be difficult to determine whether PAE is a primary contributor [6]. Nevertheless, there is increasing and compelling evidence that there is fetal programming of the hypothalamic-pituitary axis by PAE which alters its adaptability to stress and predisposes the individual to mental health disorders in later life [49].***

April has been told by her foster mother that she was diagnosed FASD. She understands from her foster mother that her biological mother loves her but had life difficulties that she coped with by using alcohol; she didn't use alcohol to hurt her. April has had multiple accommodations at home and at school to support her. While she had some challenges with learning, she had an Individualized Educational Plan with a multidisciplinary school team supporting her including the school resource and guidance teachers, and consultation from the school therapy team (speech and language therapy, occupational therapy, and physiotherapy).

*April had a close social group of friends who were supportive. She was involved with a number of after-school activities including a dance class and an art class which she enjoyed. Her parents monitored her electronic use and provided coaching regarding safe use of the internet (*good information is available at https://internetsafety101.org/agebasedguidlines).*

April had also been referred early to FASD support groups and mentors in the community. They were able to help her learn about FASD in a positive way, connect her with other individuals with FASD, and help her start planning for her future.

As April approached 18 years, her family discussed her transition to adulthood. They knew of individuals who had not received early diagnosis and who were struggling with independent living in the community or who were struggling with addictions themselves. However, April's family had planned for the transition with her primary care physician, her school, and her FASD support workers and mentors. With their support, April was able to enrol in college and was working part-time to support others with FASD. Her primary care physician continues to provide anticipatory guidance regarding her health and well-being.

Clinical Pearls:

Best practices for care across the lifespan includes anticipatory guidance at various ages on issues of medical care, mental health and well-being, and FASD education and support [6, 24].

- ***Diagnosed individuals and those that care for them should be linked to resources and services throughout the lifespan that can improve outcomes [6].***

Final Comments/Summary

An accurate and timely diagnosis for an individual with PAE experiencing neurodevelopmental difficulties at any age remains a clinical challenge but one that can positively change life outcomes for the individual and their families. Clinicians have important roles in early identification, regular surveillance where there is risk associated with PAE, and the provision of anticipatory guidance to families. Available guidelines provide a road map to understanding diagnostic criteria. Research continues to reveal novel discoveries that will improve the technologies available for screening, diagnosis and treatment. At the heart of skilled multidisciplinary assessment is the opportunity to identify the strengths of the individual and make recommendations based on the best evidence that is available. Collaboration and partnerships will improve diagnostic capacity and referral processes increasing the likelihood of positive outcomes.

Multiple Choice Questions

1. The diagnosis of FASD requires:
   a. Confirmation of prenatal drug exposure
   b. Confirmation of prenatal alcohol exposure
   c. Confirmation of maternal alcohol addiction
   d. Confirmation of maternal alcohol use
   Answer: b
2. The severity of expression of alcohol effects/FASD is influenced by:
   a. Maternal poor nutrition
   b. Genetic susceptibility
   c. Poverty and socioeconomic status
   d. All of the above
   Answer: d
3. A newborn with a confirmed history of prenatal alcohol exposure based on maternal confirmation may receive a diagnosis of FASD in which of the following situations:
   a. Well grown, term infant with jitteriness at birth
   b. Term infant with microcephaly and global developmental delay
   c. Term infant with microcephaly, sentinel facial features, and global developmental delay
   d. Well grown, term infant, with congenital cardiac anomalies
   Answer: c
4. Comprehensive multidisciplinary FASD assessment should provide recommendations that include:
   a. Strength based discussion of an individual's abilities
   b. Advocacy for appropriate services
   c. Education for the individual on their own learning styles and behavioural needs
   d. Education for family and community on the needs of the individual
   e. All of the above
   Answer: e

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23 Epidemiology of Alcohol Consumption among Pregnant and Childbearing Age Women and Fetal Alcohol Spectrum Disorder

Svetlana Popova, Jürgen Rehm, and Kevin Shield

Abbreviations

APC	Alcohol *per capita*
AD	Alcohol dependence
AUD	Alcohol Use Disorder
DALYs	Disability-Adjusted Life Years
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorder
HED	Heavy episodic drinking
PAE	Prenatal alcohol exposure
U.S.	United States
WHO	World Health Organization

Learning Objectives

By the end of this chapter, readers should be able to learn about:

- the prevalence of alcohol use and binge drinking among
  a) women of childbearing age and b) pregnant women;
- prevalence of FASD among
  b) general populations and b) specific sub-populations.

Highlights

- Globally, the prevalence of alcohol use and the volume of alcohol consumed are increasing among women of childbearing age, which lead to corresponding increases in the prevalence of women who consume alcohol during pregnancy.
- Countries with high-income economies report the greatest alcohol per capita consumption among women of childbearing age.
- FASD is prevalent alcohol-related birth defect, especially among certain sub-populations (e.g., children in care, correctional, special education, and specialized clinical facilities) and thus, it is a significant public health problem globally.
- It is crucial to improve prevention of alcohol use during pregnancy, diagnostic screening strategies, targeted interventions for women of childbearing age with substance use problems, diagnosis informed care, and the provision of support for people with FASD and their families, especially in certain sub-populations.

Prevalence estimates for a behavior, which can negatively impact health, are important tools to be used to understand the population burden of resulting diseases. Further, these estimates also reveal the scope of behavioral problems and should draw the attention of healthcare practitioners, public health authorities, policymakers, and government officials to the breadth of attendant public health problems. As such, prevalence estimates are necessary for setting public health policy priorities, securing funding for public health initiatives, planning health care strategies, allocating resources for health care and prevention, and planning and delivering health care to high-needs populations. Accordingly, this chapter reports prevalence estimates of alcohol use among childbearing-aged women (15 to 49 years of age), alcohol use during pregnancy, and Fetal Alcohol Spectrum Disorder (FASD).

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Prevalence of Alcohol Use among Childbearing-Aged Women

Data presented on alcohol consumption among women of childbearing age, and the prevalence of alcohol use disorders (AUD) and alcohol dependence (AD) among adult women (15 years of age and older), were obtained from the World Health Organization's (WHO) The Global Information System on Alcohol and Health [1]. Alcohol consumption, AUD, and AD data are presented by WHO region, as well as by World Bank income groupings.

The most accurate measure of alcohol use at the population level is adult *per capita* consumption of alcohol (APC), which is based on taxation and production statistics as well as population survey data [2]. Global APC among women of childbearing age was estimated to be 2.3 l in 2016. Worldwide, APC among women of childbearing age varied, ranging from 0.1 l in the Eastern Mediterranean region to 4.6 l in the European region. Countries with high-income economies reported the greatest APC (4.5 l), followed by upper-middle-income economies (2.7 l), lower-middle-income economies (1.5 l), and low-income economies (1.2 l) (see Table 23.1).

The ratio of men's APC to women's APC in 2016 was estimated to be 4.6 globally; highest in the Eastern Mediterranean region (10.8) and lowest in the European region (3.8). Assuming a prospective trend of convergence of gendered alcohol use [4], consumption by women is expected to continue to increase in those regions where currently there exists a large discrepancy in the amount of alcohol consumed by men versus women.

Globally, in 2016, 32.1% of women of childbearing age consumed alcohol in the past year. The prevalence of current drinking among childbearing-aged women was lowest in the Eastern Mediterranean region (1.3%) and highest in the European region (53.9%) [3].

Worldwide, approximately 8.7% of women of childbearing age engaged in heavy episodic drinking (HED), defined as consuming at least 60 grams of pure alcohol on at least one occasion in the past 30 days [5]. Of note is the definition of what constitutes a standard drink: one standard drink in the United States (U.S.) contains 14 grams of pure alcohol and one European standard drink contains approximately 10 grams of pure alcohol. The prevalence of HED among women of childbearing age was lowest in the Eastern Mediterranean region (0.1%) and highest in the European region (18.7%).

The prevalences of childbearing-aged women who consume alcohol and of this group of women who engage in HED were highest in countries with high-income economies (60.7% and 17.3%, respectively), followed by upper-middle-income economies (37.6% and 10.4%, respectively), lower-middle-income economies (20.6% and 5.0%, respectively), and low-income economies (17.4% and 5.5%, respectively).

The global prevalences of AUDs and AD among adult women were 1.7% and 0.8%, respectively, in 2016. Among adult women, the prevalence of AUDs was lowest in the Eastern Mediterranean region (0.2%) and highest in the European region (3.5%). Similarly, among adult women, the prevalence of AD was lowest in the Eastern Mediterranean region (0.1%) and highest in the European region (1.5%). Stratified by income, countries with very high-income econ-

Table 23.1 Adult *per capita* consumption and the prevalences of current drinking, former drinking, lifetime abstention, and heavy episodic drinking among women of childbearing age (15 to 49 years of age) in 2016

Regions	*Per capita* consumption (litres)	Prevalence (%)			
		Current drinking	Former drinking	Lifetime abstention	Heavy episodic drinking
Global	2.3	32.1	56.7	11.3	8.7
WHO regions					
African	2.1	21.7	69.4	9.0	7.6
America	3.1	42.9	25.0	32.0	10.5
Eastern Mediterranean	0.1	1.3	97.8	0.9	0.1
European	4.6	53.9	30.3	15.9	18.7
South-East Asia	1.4	22.5	70.5	7.0	5.2
Western Pacific	2.8	42.7	49.6	7.6	10.7
World Bank regions					
Low-income economies	1.2	17.4	73.8	8.8	5.5
Lower-middle-income economies	1.5	20.6	71.3	8.1	5.0
Upper-middle-income economies	2.7	37.6	50.7	11.8	10.4
High-income economies	4.5	60.7	18.4	20.9	17.3

Data taken from The Global Information System on Alcohol and Health [3]

omies reported the highest prevalences of AUDs and AD (5.0% AUDs and 2.4% AD), while the prevalence of AUDs was lowest in lower-middle-income economies (0.6% AUDs), and the prevalence of AD was lowest in low-income economies (0.3%).

Alcohol Use during Pregnancy

Alcohol use during pregnancy is an established risk factor for a number of adverse outcomes, including stillbirth [6], spontaneous abortion [7], premature birth [8–10], intrauterine growth retardation [10, 11], low birthweight [10, 12] and FASD [13].

Many countries educate women of childbearing age about the detrimental effects of alcohol consumption during pregnancy using clinical guidelines; examples include guidelines produced by Australia [14], Denmark [15], Canada [16], France [17], the U.S. [18], and the WHO's 2014 guidelines for the management of substance use during pregnancy [19]. Despite these public health efforts, approximately 10% of women worldwide consume alcohol during pregnancy [20].

According to a systematic literature review and meta-analysis [20], the WHO European Region was estimated to have the highest prevalence of alcohol use during pregnancy (25.2%) (see Table 23.2). This is not surprising; according to the latest Global Status Report on Alcohol and Health [3], the European region ranked highest in nearly all major alcohol indicators, including prevalences, levels of consumption, rates of alcohol use, HED, and AUDs. Additionally, the eight countries with the highest prevalences of alcohol use among pregnant women were in the European region: Ireland (60.4%), Belarus (46.6%), Denmark (45.8%), the United Kingdom (41.3%), and Russia (36.5%) [20]. The lowest prevalence of alcohol use during pregnancy was estimated to be in the Eastern-Mediterranean Region (50 times lower than the global average) followed by the South-East Asia Region (five times lower than the global average); in these regions, a large proportion of the population abstains from alcohol use, especially within the female population [20].

In addition to alarmingly high rates of alcohol consumption during pregnancy, it has also been estimated that more than 25% of the women who consume any alcohol during pregnancy also engage in binge drinking, defined as consuming four or more drinks on a single occasion [21, 22]. This is very troubling, as binge drinking is the most detrimental pattern of drinking during pregnancy and is a direct cause of FASD. The highest prevalence of binge drinking during pregnancy was estimated to be in the African region (3.1%), while the lowest prevalence of binge drinking during pregnancy was estimated to be in the Western-Pacific region (1.8%) (see Table 23.2).

Among women who drank any amount of alcohol during pregnancy, the proportion who engaged in binge drinking was estimated to range from 10.7% in the European region to 31.0% in the African region [21]. The five countries with the highest estimated prevalences of binge drinking during pregnancy were Paraguay (13.9%), Moldova (10.6%), Ireland (10.5%), Lithuania (10.5%), and the Czech Republic (9.4%) [21]. The prevalence of women who consumed alcohol during pregnancy (among the five countries with the highest estimated prevalences of binge drinking during pregnancy) was Paraguay (77.7%), Benin (77.2%), Seychelles (77.2%), Austria (59.5%), and Zimbabwe (52.5%) [21, 22].

The estimates presented in this section are representative of the general populations of the respective countries. However, prevalence rates of alcohol use during pregnancy have been reported to be much higher among some subpopulations. For example, the prevalence of alcohol use dur-

Table 23.2 Global prevalences of any amount of alcohol use and binge drinking (4 or more drinks on a single occasion) during pregnancy, and of FAS and FASD among the general population, by WHO region, and corresponding 95% confidence intervals

Region	Alcohol use(any amount) during pregnancy (%)[a]	Binge drinking during pregnancy (%)[b]	FAS(per 10,000)	FASD(per 10,000)[c]
Globally	9.8(8.9,11.1)	–	9.4(9.4,23.3)	77.3(49.0,116.1)
WHO regions				
African	10.0(8.5,11.8)	0.1(0.1,6.1)	8.9(8.9,21.5)	78.3(53.6,107.1)
America	11.2(9.4,12.6)	0.1(0.1,5.6)	11.0(11.0,24.0)	87.9(63.7,132.4)
Eastern Mediterranean	0.2(0.1,0.9)	–	0.2(0.2,0.9)	1.3(0.9,4.5)
European	25.2(21.6,29.6)	0.0(0.0,5.3)	24.7(24.7,54.2)	198.2(140.9,280.0)
South-East Asia	1.8(0.9,5.1)	–	1.3(1.3,8.1)	14.1(6.4,53.1)
Western Pacific	8.6(4.5,11.6)	0.0(0.0,3.5)	7.7(7.7,19.4)	67.4(45.4,116.6)

Reproduced with permission from Popova et al. [21]

[a]The prevalence of any amount of alcohol use during pregnancy is inclusive of the prevalence of binge drinking during pregnancy

[b]It was not possible to estimate the prevalence of binge drinking during pregnancy for the Eastern-Mediterranean or South-East Asia regions due to a lack of available data for countries in these regions; therefore, the global prevalence could not be estimated

[c]The prevalence of FASD includes the prevalence of FAS

ing pregnancy among Inuit women in northern Quebec (Canada) was reported to be 60.5%, which is over ten times higher than the estimate for the general population of Canada [23].

What Is Fetal Alcohol Spectrum Disorder?

Alcohol is a teratogen that can readily cross the placenta, resulting in the central nervous system and physical damage to the developing embryo and fetus, and which also may lead to FASD. FASD is an umbrella term describing a wide range of effects, including central nervous system damage, congenital anomalies, prenatal or postnatal growth restrictions, characteristic dysmorphic facial features, and deficits in cognitive, behavioral, emotional, and adaptive functioning. Depending on the classification system, FASD includes several alcohol-related diagnoses, such as fetal alcohol syndrome (FAS)—which is the most severe form of FASD, partial FAS (pFAS), alcohol-related neurodevelopmental disorder (ARND), and Alcohol-Related Birth Defects (ARBD) [24, 25]. The Canadian framework utilizes a single designation of FASD as a diagnostic term, with the specification of the presence or absence of the characteristic facial features [26]. In addition, the category of neurodevelopmental disorder associated with prenatal alcohol exposure (ND-PAE) is included in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5; [27]).

FAS was first described in the French medical literature by Paul Lemoine and colleagues in a 1968 study of children born to alcohol-dependent parents [28]. Five years later, Ken Jones and David Smith published a paper in *The Lancet* describing the association between alcohol abuse and morphological defects and provided diagnostic criteria for this condition [29, 30]. These publications helped to define the morphological defects, developmental delays, and growth deficits of children born to mothers with confirmed alcohol consumption during pregnancy. Since FAS was first described, several classification systems have been developed to standardize and clarify the spectrum of impairments caused by prenatal alcohol exposure (PAE). Although existing guidelines thoroughly describe FASD diagnostic criteria, issuing a diagnosis of FASD remains challenging for clinicians as the specific assessment techniques used to make a definitive diagnosis are still under debate.

FASD has a very broad phenotype and is further complicated by high rates of comorbidity. More than 400 disease conditions have been reported to co-occur in people diagnosed with FASD [31]. The most prevalent conditions that occur in individuals with FASD are found within the categories of congenital malformations, deformities, and chromosomal abnormalities (43%) and mental and behavioral disorders (18%) (see chapters of the International Statistical Classification of Diseases and Related Health Problems, tenth Revision (ICD-10) [32]. Some comorbid conditions that are highly prevalent among people with FASD include language, auditory, visual, developmental, cognitive, mental, and behavioral issues, with prevalence estimates of these conditions ranging from 50% to 91% [31].

The neurodevelopmental impairments associated with PAE lead to other disabilities later in life. These disabilities include, but are not limited to, academic failure, substance abuse, mental health problems, contact with law enforcement, and an inability to live independently and obtain and/or maintain employment—all of which have lifelong implications [33].

Individuals from all socio-economic and ethnic backgrounds are affected by FASD [34]. Moreover, FASD is an intergenerational issue, and children with an affected sibling are at a higher risk of having FASD [35].

The complexity and chronicity of FASD affects both the affected individual and their family. In many cases, people with FASD require lifelong support from a wide range of support services, including health care, social assistance, and remedial education. Accordingly, it has been shown that FASD has a substantial economic impact on any society [36, 37]. In North America, the lifetime cost for a complex case of FASD has been estimated to be more than $1 million CAD [38].

Research in humans has not yet determined the pattern, amount, or critical period of prenatal alcohol exposure necessary for structural or functional teratogenesis. What is known is that not every child with PAE will be diagnosed with FASD. It is hypothesized that there may be other factors influencing the vulnerability of a fetus to the teratogenic effects of alcohol. Some factors include variability in the metabolism and genetic background of both the mother and fetus, environmental influences, maternal smoking behaviors, nutritional status, stress levels [39, 40], and possibly paternal lifestyle [41]. It has been estimated that approximately one in every 13 prenatally alcohol-exposed infants will have FASD, which would result in approximately 630,000 infants being born with FASD worldwide each year [42].

Prevalences of Fetal Alcohol Syndrome (FAS) and Fetal Alcohol Spectrum Disorder (FASD) in the General Population

Prevalences of FAS and FASD were estimated to be highest in the European region, at 37.4 per 10,000 people and 198.2 per 10,000 people, respectively, and lowest in the Eastern Mediterranean region, at 0.2 per 10,000 people and 1.3 per

10,000 people, respectively [20, 42]. These estimates are in line with the above-reported prevalences of alcohol use during pregnancy. Globally, prevalence rates of FAS and FASD among the general population were estimated to be 14.6 per 10,000 people and 77.3 per 10,000 people, respectively [20, 42]. The five countries with the highest prevalence of FAS were Belarus (69.1), Italy (82.1), Ireland (89.7), Croatia (115.2), and South Africa (585.3) [20]. Conversely, the five countries with the lowest prevalence of FAS per 10,000 people (less than 0.05) were Oman, United Arab Emirates, Saudi Arabia, Qatar, and Kuwait, all of which countries are found in the Eastern Mediterranean Region) [20]. Similarly, the five countries with the highest prevalence of FASD per 1000 were South Africa (111.1), Croatia (53.3), Ireland (47.5), Italy (45.0), and Belarus (36.6) [42]. The five countries with the lowest prevalence of FASD (<0.1 per 1000) were Oman, United Arab Emirates, Saudi Arabia, Qatar, and Kuwait [42].

The prevalence of FASD in the Western Cape Province of South Africa, a region known for wine production and a high prevalence of binge drinking among women, has been reported to be 135.1 to 207.5 per 1000 (13.5–20.8%) among first grade students—one of the highest FASD prevalence rates in the world [43].

FASD Prevalence in Sub-Populations

A systematic review and meta-analysis revealed that certain sub-populations experience a significantly higher prevalence of FASD compared to the general population [44]. These sub-populations include children in care, correctional, special education, and specialized clinical facilities, and in Aboriginal populations (see Fig. 23.1). These findings demonstrate the large service and cost burdens of FASD across various systems of care and reflect a substantial global health problem [44].

The pooled prevalence of FASD among children in out-of-home care/foster care was estimated to be 31.2% in Chile and 25.2% in the U.S. [44]. The pooled prevalence of FAS was estimated to be 9.6% among children in care in Russia [44]. The pooled FASD prevalence among Aboriginal populations was estimated to be 4.4% and among adults in the Canadian correctional system, the pooled FASD prevalence was estimated to be 14.7% [44].

Prevalence estimates of FASD among these sub-populations far exceed those seen in the general population. Compared to a global prevalence estimate of 7.7 FASD cases per 1000 people in the general population [42], prevalences in the selected sub-populations were 10 to 40 times higher.

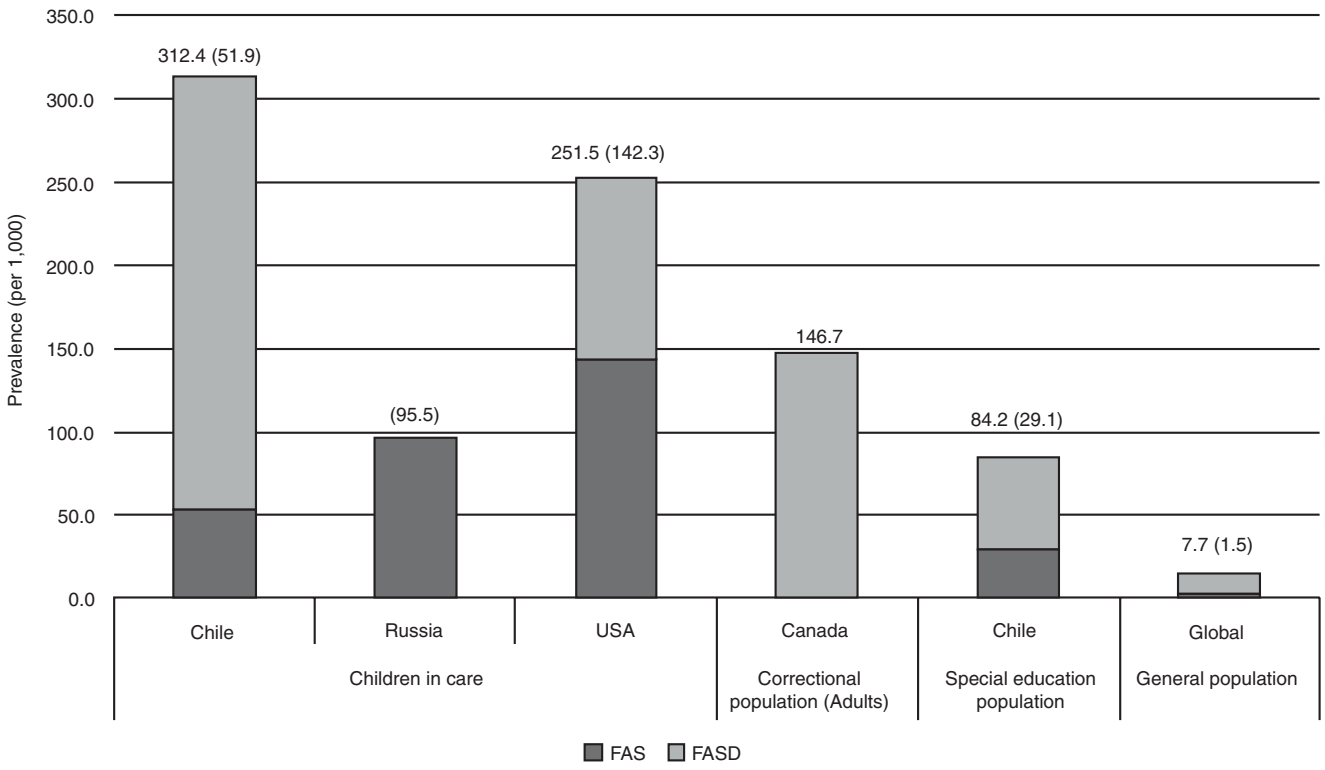

Fig. 23.1 Pooled prevalences of FAS and FASD among selected sub-populations, by country and in the general global population. *Note: FAS prevalence is in brackets*

For example, compared to the general population, the prevalence of FASD among children in care was 32 times higher in the U.S. and 40 times higher in Chile, the prevalence of FASD among adults in the Canadian correctional system was 19 times higher, and the prevalence of FASD among special education populations in Chile was over 10 times higher [44].

Furthermore, prevalence rates reported in individual studies (some not meta-analyzed) are even higher and more alarming. For instance, the prevalence of FASD was 62% among children with intellectual disabilities in care in Chile [45], over 52% among adoptees from Eastern Europe [46], and approximately 40% among children residing in orphanages in Lithuania [47]. The highest prevalence estimates of FAS, which ranged between 46% and 68%, were reported in Russian orphanages for children with developmental abnormalities [48]. Additionally, the prevalence of FASD among youth in correctional services was over 23% in Canada [49], and over 14% among U.S. populations in psychiatric care [50]. Children are often placed in foster care, orphanages, or for adoption due to unfavorable circumstances, such as parental alcohol and/or drug use, child maltreatment, abandonment, and young maternal age (see Chap. 23). These circumstances suggest a higher likelihood of PAE among children in care [51].

Appropriate social work interventions, as well as diagnostic and support services, must be available to individuals with FASD from an early age in order to decrease their chances of becoming involved with the legal system, whether as a victim or as an offender. One estimate suggests that, on any given day in a specific year, children and adolescents with FASD are 19 times more likely to be incarcerated compared to children and adolescents without FASD [52]. Lastly, high prevalence rates of FASD among special education and specialized clinical populations are not surprising given that individuals with FASD are at an increased risk of having learning difficulties and mental health problems, and of experiencing developmental delays [31].

High prevalence rates of alcohol consumption during pregnancy and FASD within Aboriginal populations must be examined within the historical context and socio-demographic realities of these marginalized sub-populations, including colonization, intergenerational trauma, and residential school experiences. These factors contribute to the high prevalence of alcohol use, both in general and during pregnancy [53, 54]. For example, Aboriginal populations in the U.S. and Canada reported prevalence rates of alcohol use during pregnancy that were approximately 3 and 4 times higher, respectively, than the general population [55]. Even more alarming, among Aboriginal women in the U.S. and in Canada who consumed alcohol during pregnancy, approximately 20% engaged in binge drinking compared to 3% in the general population in both countries [55–58].

Conclusion

The harmful effects of alcohol on a fetus, which lead to many cases of preventable long-term disability, should be recognized globally as a substantial public health problem. Until now, prevalence estimates of alcohol use and binge drinking during pregnancy, as well as estimates of the prevalences of FAS and FASD, have been unavailable for the majority of countries. Unfortunately, this lack of information has influenced the prioritization of healthcare expenditures for those populations most at-risk. The figures presented above are alarming as they reveal the far-reaching nature of these harmful effects and why urgent action is required.

The above-stated findings also emphasize that FASD is not restricted solely to disadvantaged groups, but rather that it occurs throughout society, regardless of socio-economic status, education, or ethnicity. Therefore, efforts must be made to better educate the general population (adult women and men, children, and teenagers) about the risks of alcohol use (especially binge and frequent drinking) during pregnancy. Moreover, prevention initiatives aimed at reducing alcohol use prior to and during pregnancy should be implemented worldwide. Appropriate screening for alcohol use in all women of childbearing age, in combination with health promotion before conception, contraceptive counselling, brief interventions, and referrals to substance abuse programs where appropriate, should become a routine standard of care. Referrals to substance abuse programs, if necessary, are of the utmost importance; effective treatment of identified cases of AD or AUDs could reduce the risk of having a child with FASD. Healthcare providers and other professionals are in a position to fulfil a crucial role in the primary prevention of PAE and FASD. Finally, as they are established public health problems, all countries should develop surveillance systems to monitor the prevalence of alcohol use during pregnancy and of FASD, over time, to identify vulnerable populations, develop resources, and evaluate the effectiveness of prevention and research strategies.

Multiple Choice Questions

1) Which WHO region has the highest prevalence of alcohol use among childbearing age women?
   a) African
   b) American
   c) Eastern Mediterranean
   d) European
   e) South-East Asia
   f) Western Pacific
   Answer: d) European WHO Region
2) Which WHO region has the highest prevalence of FASD in the general population?
   a) African
   b) American

c) Eastern Mediterranean
d) European
e) South-East Asia
f) Western Pacific

Answer: d) European WHO Region

3) A high proportion of people with FASD have trouble with the law because they (check all that apply)
   a) exhibit impulsive behaviours
   b) frequently aim to please others, which makes them vulnerable to being misled by others
   c) have a poor understanding of societal norms and expectations
   d) have learning difficulties
   e) have language difficulties
   f) have difficulty in learning from past experiences
   g) all of the above

   Answer: g) all of the above
4) Some women consume alcohol during pregnancy (even in binges) and their children do not have FASD

   True
   False

   Answer*:* True
5) Select reason(s) for the increasing prevalence of FASD in the world in the near future
   a) the rates of alcohol use are increasing among young women in a number of countries
   b) binge drinking is increasing among young women in a number of countries
   c) drinking during pregnancy is increasing among young women in a number of countries
   d) a vast majority of pregnancies are unplanned
   e) all of the above

   Answer: e) all of the above

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Fetal Alcohol Spectrum Disorder: Diagnosis

24

Jocelynn L. Cook and Courtney R. Green

Learning Objectives

- To identify the range of effects from prenatal alcohol exposure
- To understand the criteria required for an FASD diagnosis
- To explore challenged with FASD diagnosis

Highlights

- The diagnosis of FASD is complex and requires a highly trained, multidisciplinary team including physicians, psychologists and other allied health care professionals.
- An FASD diagnosis is based on a combination of factors including central nervous system problems (structural, neurological, and/or functional), facial dysmorphology (smooth philtrum, thin upper lip, and short palpebral fissure) and confirmed prenatal alcohol exposure.
- There is no safe threshold for alcohol consumption during pregnancy.
- All pregnant women and women of child-bearing age should be asked periodically about alcohol, tobacco, prescription drug use, and illicit drug use, and those at- risk for problematic substance use should be offered brief interventions and referral to community resources.
- A disproportionate number of individuals with FASD have mental health comorbidities, such as depression; mood and anxiety disorders; attention deficit /hyperactivity disorder (ADHD); and conduct disorder.

Introduction

Prenatal exposure to alcohol can lead to harmful developmental outcomes and is the leading known cause of developmental disability in the western world. Fetal Alcohol Spectrum Disorder (FASD) describes the constellation of adverse effects that can result from maternal consumption of alcohol during pregnancy. The cognitive and behavioural symptoms associated with FASD are both debilitating and life-long [1]. The extent and severity of these deficits require a high level of coordinated service delivery that is extremely costly to the health care system. FASD is not unique to developed countries and is a growing global health concern worldwide.

The diagnosis of FASD is complex and requires a highly trained, multidisciplinary team including physicians, psychologists and other allied health care professionals. This level of expertise is essential to the accurate diagnosis of FASD-related disabilities and for identifying functions and deficits—especially neurodevelopmental—and for recommending interventions that will not only improve outcomes for affected individuals and their families but also provide access to services.

Diagnosis is based on a combination of factors including central nervous system problems (structural, neurological,

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and/or functional), facial dysmorphia (smooth philtrum, thin upper lip, and short distance between the inner and outer corners of the eyes) and confirmed prenatal alcohol exposure. The confirmed absence of alcohol during pregnancy rules out a diagnosis of FASD. Diagnostic practices vary depending on the diagnostic schema used; clinical categorization is not standardized [2, 3].

FASD can be diagnosed at birth, but often goes undiagnosed until later in life when behavioural and cognitive effects become more evident [4]. Early diagnosis (e.g., before age 6 years) and intervention are considered critical to improve development and to reduce the likelihood of secondary disabilities [5, 6].

Diagnosis can be extremely complicated and, in an attempt to guide diagnostic teams, a number of approaches have been identified around the world. *Fetal Alcohol Spectrum Disorder: Canadian Guidelines for Diagnosis* [7] were published by a panel of Canadian experts in 2005 in an attempt to provide simple, but thorough, evidence-based guidelines for diagnoses related to prenatal exposure to alcohol. These Canadian guidelines were updated in 2016 [1]. Additional diagnostic guidelines include those from the Institute of Medicine [8], the FASD 4-Digit Diagnostic Code [9], the Centers for Disease Control and Prevention FAS guidelines [10], the updated Hoyme FASD guidelines [11] and the Mortality and Morbidity Weekly Report Guidelines [12]. Several countries have used these different schemas to inform the developmental of their own diagnostic guidelines, for example, Argentina [13], Netherlands [14] Germany [15], Australia [16], and Scotland [17].

There is consensus among the diagnostic schemas that the facial features associated with prenatal alcohol exposure are unique to FASD; however, they differ in the number of features that must be present to obtain a diagnosis. It is the central nervous system dysfunction that has the most diverse range of potential deficits and variability, which leads to differences in the final diagnosis. A critical implication of this predicament is the inability to combine data from sources that use different diagnostic schemas preventing the creation of large datasets that can be used for research, to calculate prevalence and inform nomenclature.

Coles et al. [18] compared the most common diagnostic schema for FASD and suggested problems in convergent validity among systems, as demonstrated by a lack of reliability in diagnosis, but concluded that the absence of an external standard makes it impossible to determine whether any system is more accurate. Table 24.1 illustrates the differences between systems.

Currently, diagnosis of FASD continues to be problematic depending on a variety of reasons including lack of diagnostic capacity, lack of antenatal history and lack of support. These reasons further highlight the need to create comprehensive diagnostic guidelines that can be implemented easily, with as much objectivity as possible. Clearly, diagnosis has significant implications for incidence, prevalence and surveillance, economic and cost calculations, and supply and demand of services across many sectors. For the purposes of this textbook, the 2016 Canadian Guidelines will be used [1], recognizing that, while using different systems may result in different specific diagnoses, the process of physical and neurodevelopmental assessment is largely the same.

In 2005, *Fetal Alcohol Spectrum Disorder (FASD): Canadian Guidelines for Diagnosis* [7] was published as a supplement to the Canadian Medical Association Journal. The guidelines were created following widespread consultation with expert practitioners and partners in the field and were the first attempt to provide user-friendly, evidence-based guidelines for diagnostic services related to Fetal Alcohol Spectrum Disorder (FASD) in the Canadian context. Eventually, more experience revealed gaps and inconsistencies in areas that needed improvement and/or clarity (i.e. diagnostic recommendations specific for very young children and adults); and, following widespread consultation with experts from around the world, the Canadian guidelines were updated in 2016 [1].

Table 24.1 Comparisons of the most commonly used diagnostic systems

Diagnostic System	Prenatal Exposure	Dysmorphic Features	Growth	Neurodevelopment	FAS	Partial FAS	ARND	Comments
Canadian 2016	Confirmation of prenatal alcohol exposure is required, with the estimated dose at a level known to be associated with neurobehavioural effects	• Presence of 3 facial features; palpebral fissure length ≤ third percentile (2 SD below mean); lip and philtrum ranked 4 or 5.	• Documented but not included in diagnostic criteria	• 3 brain domains of impairment: – Motor skills – Neuroanatomy / neurophysiology – Cognition – Language – Academic achievement – Memory – Attention – Executive function, including impulse control – Affect regulation – Adaptive behaviour, social skills, or social communication – Scores on standard measures less than or equal to 2 standard deviations (SD) below the mean, or less than or equal to the third percentile indicate significant impairment.	• Terminology is "FASD with sentinel facial features" • 3 facial features present • 3 brain domains of impairment • Does not require confirmation of prenatal alcohol exposure	• Terminology is "FASD without sentinel facial features" • <3 facial features present • 3 brain domains of impairment • Requires confirmation of prenatal alcohol exposure	• Terminology is "FASD without sentinel facial features" • <3 facial features present • 3 brain domains of impairment • Requires confirmation of prenatal alcohol exposure	• "At risk" designation for those who don't yet meet criteria but may in the future
Emory FAS Clinic	• Documents mother's alcohol consumption patterns during pregnancy. • Does not accept "hearsay" evidence	• Uses weighted scoring system to characterize dysmorphic features. Uses "10" and "20" as cutoff score for diagnosis vs "sentinel features". • Features based on Jones and Smith (1973) and results from longitudinal exposure sample	• Weight or length/ height ≤ tenth percentile at birth or diagnosis • Must rule out other causes for growth retardation.	• Head circumference ≤ tenth percentile under 3 years or medical signs • Cognitive deficits based only on developmental and cognitive testing. • Behavioural reports/ checklists not accepted		• Requires confirmation of prenatal alcohol exposure • Requires 2 of the other 3 criteria (not necessarily the 3 facial features)	• Requires confirmation of prenatal alcohol exposure • Cognitive deficits without physical features	

(continued)

Table 24.1 (continued)

Diagnostic System	Prenatal Exposure	Dysmorphic Features	Growth	Neurodevelopment	FAS	Partial FAS	ARND	Comments
CDC and FAS task force	• Confirmed prenatal alcohol exposure • Unknown prenatal alcohol exposure	• Presence of the 3 sentinel facial features	• Weight or length and/or height ≤ tenth percentile at any time	• Head circumference ≤ tenth percentile • Neurological signs. • Functional deficits in developmental and cognitive testing, either globally (< 2SD) or in 3 areas of development (<1 SD).		• Not enough scientific evidence for creation of other diagnostic categories	Not included	
Hoyme 2016	• Prenatal alcohol exposure documented • Information must be Obtained from the biological mother or a reliable collateral source • Prenatal alcohol exposure not documented	• Presence of ≥2 facial features; palpebral fissure length ≤ tenth percentile; lip and philtrum ranked 4 or 5.	Height and/or weight ≤ tenth percentile	• Deficient brain growth, abnormal morphogenesis, or abnormal neurophysiology, including ≥1 of the following; head circumference ≤ tenth percentile, structural brain anomalies, recurrent nonfebrile seizures • Neurobehavioral impairment (cognitive OR behavioral impairment) • In children <3 yrs., evidence of developmental delay ≥1.5 SD below the mean	• Prenatal alcohol exposure documentation not required • Presence of ≥2 facial features • Neurobehavioral impairment (cognitive OR behavioral impairment) • Height and/or weight ≤ tenth percentile • Small head circumference (≤tenth centile)	• With confirmation of prenatal alcohol exposure: • Presence of ≥2 facial features; palpebral fissure length ≤ tenth percentile; lip and philtrum ranked 4 or 5. • Neurobehavioral impairment (cognitive OR behavioral impairment) • In children <3 yrs., evidence of developmental delay ≥1.5 SD below the mean • Without confirmation of prenatal alcohol exposure: • Presence of ≥2 facial features; palpebral fissure length ≤ tenth percentile; lip and philtrum ranked 4 or 5. • Neurobehavioral impairment (cognitive OR behavioral impairment) Growth deficiency (height and/or weight ≤ tenth centile) OR small head circumference (≤tenth centile)	• Requires confirmation of prenatal alcohol exposure • Neurobehavioral impairment (cognitive OR behavioral impairment) • Cannot be made in ≤3 yrs	Includes ARBD: • Requires confirmation of prenatal alcohol exposure • One or more specific major malformations demonstrated in animal models and human studies to be the result of prenatal alcohol exposure
4-digit system	• Rankings based on risk of exposure from level 1 (no risk, confirmed lack of exposure) to 4 (confirm3ed high levels of exposure)	• Presence of the 3 sentinel facial features; palpebral fissure length 2 SD below mean; lip and philtrum ranked 4 or 5.	• Range from weight and height/ length ≤ tenth percentile (rank 2) to ≤second percentile (rank 4)	• For a "4", must have head <third percentile. Also seizure disorder, abnormal brain structure or IQ ≤70. • For other ranks, 3+ domains of behavior at >2 SD below the mean.	• Growth 2, 3, or 4 • Face 4 • Brain 3 or 4 • Prenatal alcohol exposure 2, 3 or 4	• Produces 9 categories, from no effects to FAS. • Face 1, 2 or 3 • Brain 3 or 4 • Prenatal alcohol exposure 3 or 4 • ~static encephalopathy—Alcohol exposed or sentinel physical findings static encephalopathy—Alcohol exposed	Includes similar categories without attributing the effects to alcohol (e.g., neurobehavioral disorder-alcohol exposed)	

Epidemiology and Cost of FASD

The specific prevalence of FASD is the subject of a number of investigations around the world. Lange *et al* estimate that the global prevalence of FASD is 7.7 per 1000 population, with some countries being as high as 111 per 1000 [19]. These statistics continue to be difficult to obtain, due to the absence of a centralized database in which diagnostic data could be entered and tracked.

The rate of FASD in the United States has been estimated at 15 per 1000 live births [20]. Prevalence data for FASD in Canada were reported at 5 per 1000 live births [20], with rates up to 16 times higher among some populations. Of the statistics that are available, it is likely that they are an underestimate of the actual prevalence in most countries around the world, due in part to delayed diagnosis, misdiagnosis or no diagnosis at all. For example, underestimates of the prevalence of FASD have been reported in communities in Italy [21] and in Western Australia due to limitations in the birth defect registry (birth defects are only recorded for children between 0–1 year of age) [22] Without accurate prevalence data, it is difficult to determine total cost (direct and indirect) associated with FASD. Greenmyer et al. [23] suggest that the mean annual cost for children with FASD is estimated at $22,810 and for adults $24,308, but conclude that the data on the economic burden of FASD are scarce, and the existing estimates likely underestimate the full economic impact on affected individuals, their caregivers, and society.

The economic burden of FASD in Canada, specifically, was reported to total approximately $1.8 billion (from about $1.3 billion as the lower estimate up to $2.3 billion as the upper estimate) in 2013 [24]. The highest contributors to the economic burden were costs associated with productivity losses (due to morbidity and premature mortality), the cost of corrections, and the cost of health care.

Importance of Early Diagnosis

Although a substantial amount of research has been devoted to furthering the understanding of prenatal alcohol pathophysiology, prevention continues to be an ongoing challenge. FASD is among the leading, preventable causes of developmental disability and a major public health concern. The early recognition of at-risk children is critical for initiating the appropriate intervention and treatment strategies. Qualitative data reveals that the "lived" experiences of parenting a child with FASD include a range of concerns, such as societal, educational, health and judicial [25]; all of which can be significantly impacted by the time a diagnosis is made. Women expressed great difficulty in obtaining a diagnosis for their child from a medical professional and frustration with the process, as they felt that they were constantly dealing with barriers and challenges with the system [25].

Infants who were non-dysmorphic at birth were later found to be at the greatest risk for significant behavioural problems as children and adolescents [26]. Without the distinct facial presentation, FASD diagnosis at birth continues to remain elusive; though no less important [27]. The need for a concerted and coordinated effort, to provide the appropriate education to practicing physicians about the phenotypes of FASD, must be made to ensure accurate and timely diagnoses; only then can the most appropriate management plans be put in place [28, 29].

Background and Terminology for the Diagnosis of Fetal Alcohol Spectrum Disorder

In 1968, Lemoine published a seminal paper that first identified the relationship between prenatal alcohol exposure and birth defects [30]. Although his initial observations did not attain international resonance, a few years later several physicians published the first case studies in North America describing the physical findings of babies born to chronic alcoholic mothers [31, 32] and coined the term "Fetal Alcohol Syndrome" (FAS). In 1978, four post-mortem anatomical assessments were conducted and profound neurological malformations stemming from errors in the migration of neuronal and glial elements [33]. Slowly the medical community began to accept that alcohol was a teratogen. Since the first description of FAS, a number of different terminologies have been introduced and revised. Still, FAS continues to be a diagnostic term that is used today and can be recognized on both a national and international scale. Interested readers are referred to Calhoun and Warren's review for a historical account of the evolution of FAS as a medical diagnosis [34].

Terminology Associated with Diagnosis

Because terminology is tightly associated with diagnostic criteria, several different approaches have been developed along with their own specific nomenclature to describe the different manifestations of prenatal alcohol exposure (Tables 24.1 and 24.2). A number of diagnostic guidelines for FASD have been published; most notably those by the Institute of Medicine (IOM) FASD [8], the FASD 4-Digit Diagnostic Code [9], the Centers for Disease Control and Prevention (CDC) FAS guidelines [10], the Canadian FASD guidelines [1], the Hoyme FASD guidelines [11] and the Mortality and Morbidity Weekly Report Guidelines [12]—all of which

Table 24.2 Compares the terminology with that of the 2016 Canadian Guideline: [With permission from the CMAJ, Reference 1]

2016 Canadian Guidelines	FASD with Sentinel Facial Features	FASD without Sentinel Facial Features	At Risk for Neurodevelopmental Disorder and FASD, Associated with Prenatal Alcohol Exposure
2005 Canadian diagnostic guidelines	FAS	pFAS ARND	
Institute of Medicine (IOM)	FAS pFAS	ARND	
4-digit diagnostic code (4DDC)	Face 4 Brain 3 or 4 PAE 2, 3 or 4 FAS	Face 1, 2 or 3 Brain 3 or 4 PAE 3 or 4 ~SE-AE or sentinel physical findings SE-AE	Face 1, 2, 3 or 4 Brain 2 (or untestable at time of assessment) PAE 2 (for face 4 ~ NB-AE), 3 or 4
Standard protocol developed by the collaborative initiative on fetal alcohol Spectrum disorders (Hoyme)	FAS with/without confirmed maternal alcohol exposure	Partial FAS with/ without confirmed maternal alcohol exposure ARND	
Diagnostic and statistical manual of mental disorders—5 (DSM-5)	315.8 neurodevelopmental disorder, associated with prenatal alcohol exposure Neurobehavioral disorder, associated with prenatal alcohol exposure (appendix 3[s])	315.8 neurodevelopmental disorder associated with, prenatal alcohol exposure Neurobehavioral disorder, associated with prenatal alcohol exposure (appendix 3[s])	
Centers for Disease Control and Prevention (CDC)	FAS		

ARND Alcohol-Related Neurodevelopmental Disorder; *FASD* Fetal Alcohol Spectrum Disorder; *FAS* Fetal Alcohol Syndrome; *pFAS* partial Fetal Alcohol Syndrome; *NB-AE* Neurobehavioral Disorder–Alcohol Exposed; *SE-AE* Static Encephalopathy–Alcohol Exposed

share similar diagnostic approaches and in the case of the 4-digit diagnostic code include a number of additions. To date, several comparisons have been made in the literature [18, 35] (Astley et al., 2006; Coles et al., 2016) and the 2005 Canadian guidelines published a harmonization of the 4-digit diagnostic code and IOM [7]. Regardless of the differences in terminology, an important evolution of all diagnostic approaches is the move towards providing services based on an individual's needs, and not on what caused their diagnosis. Specifically, it is recommended that services should be available for individuals across the full continuum of FASD—for this reason, the 2016 Canadian Guidelines added the "At-Risk" designation in recognition of this need [1]. Regardless, it is critical to remember, while the diagnostic features associated with FASD represent a spectrum of effects, the specific diagnostic terms are not indicative of a continuum of severity of the neurodevelopmental deficits.

The term FASD was originally coined as an umbrella term to encompass the diagnoses and the breadth of disabilities associated with prenatal alcohol exposure. Commonly used terminologies include: Fetal Alcohol Syndrome (FAS), Fetal Alcohol Effects (FAE), Partial Fetal Alcohol Syndrome (pFAS), Alcohol-Related Neurodevelopmental Disorder (ARND), Alcohol-Related Birth Defects (ARBD), the 4-Digit Diagnostic Code nomenclature: Neurodevelopmental Disorder, associated with Prenatal Alcohol Exposure (ND-PAE), Neurobehavioral Disorder—Alcohol Exposed (NB-AE), and Static Encephalopathy—Alcohol Exposed (SE-AE). The terminology can be confusing and the categories overlap in some instances (Table 24.1).

The Canadian Guideline attempted to simplify diagnosis into FASD with or without sentinel facial features and the "at risk" designation [1], and suggests the following, based on experience and expert opinion:

> FASD should now be used as a diagnostic term when prenatal alcohol exposure is considered to be a significant contributor to observed deficits that cannot be explained by other etiologies. Because the observed deficits are recognized as being multifactorial in origin, all other known relevant contributors (e.g., trauma, known genetic anomalies) should be documented with the FASD diagnosis as they have significant impact on the functional and neurological challenges of the affected individuals [1].

Diagnosis

A summary of the criteria for diagnosis based on the Canadian Guidelines is shown in Table 24.3 and the pathway for achieving each diagnosis (or for a non-FASD diagnosis) is shown in Fig. 24.1.

Table 24.3 Diagnostic Criteria from Canadian Guideline

	FASD with Sentinel Facial Features	FASD without Sentinel Facial Features	At Risk for Neurodevelopmental Disorder and FASD, Associated with Prenatal Alcohol Exposure
Confirmation of prenatal alcohol exposure	Not required	Required	Required
Face	3 facial features	No facial features required	No facial features required
Brain	3 domains of impairment (or microcephaly for infants)	3 domains of impairment	At least 1 domain of impairment

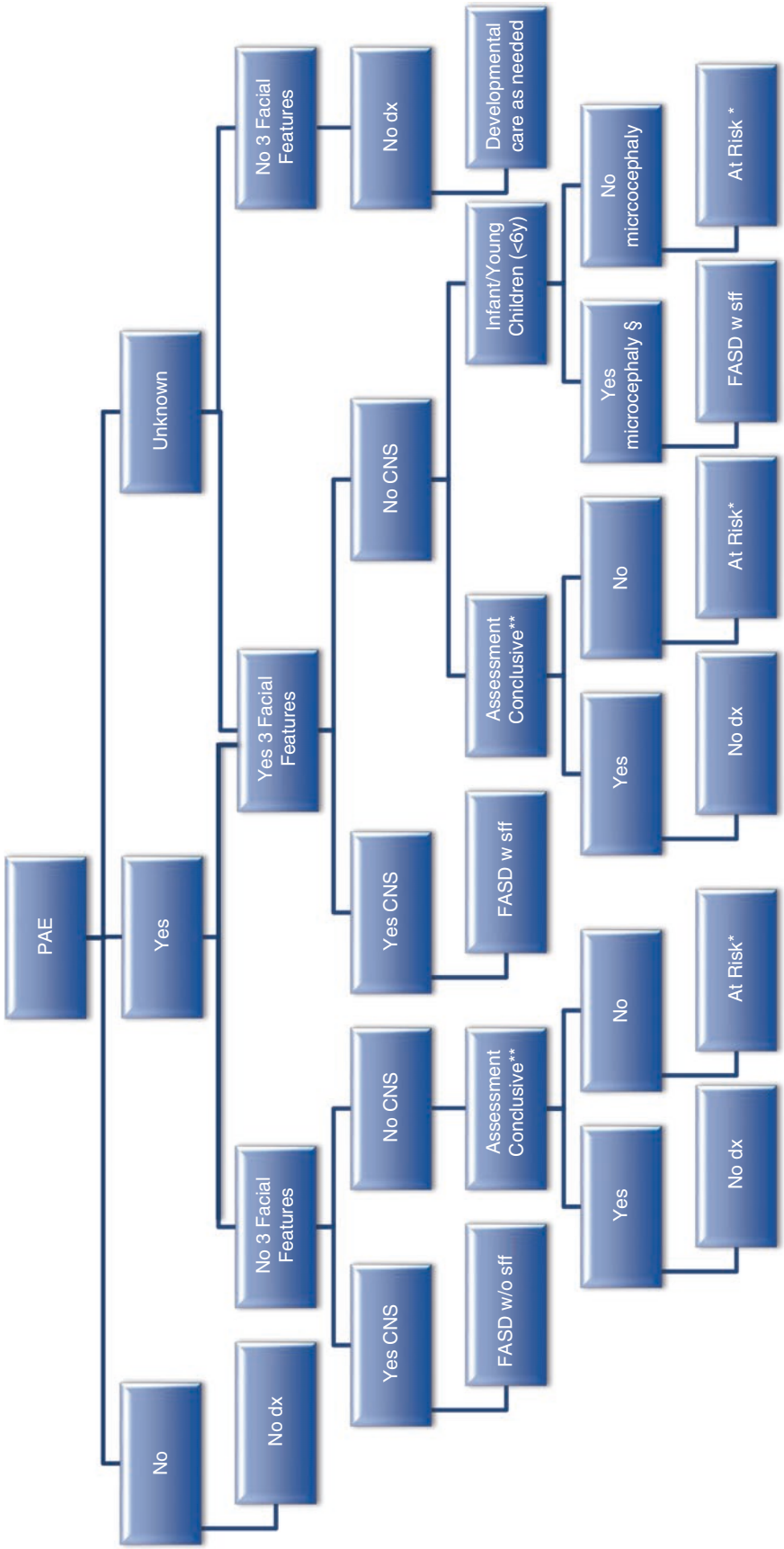

Fig. 24.1 Algorithm from Canadian Guideline [With permission from the CMAJ, Reference 1]

The Diagnostic Process

Confirmation of Prenatal Alcohol Exposure

Confirmation of prenatal alcohol exposure (PAE) requires documentation that the biological mother consumed alcohol during the index pregnancy based on: reliable clinical observation; self-report; reports by a reliable source; medical records documenting positive blood alcohol concentrations; alcohol treatment or other social, legal or medical problems related to drinking during the pregnancy [1].

Prenatal Alcohol Exposure and the Developing Fetus

In the absence of facial dysmorphology, confirmed prenatal alcohol exposure is needed to identify FASD. However, in many cases, the antenatal records are unavailable making it impossible to determine the precise dosimetry data for the timing and dose of alcohol in pregnancy. That said, several features of alcohol metabolism are well characterized, have significant implications for the developing fetus, and are important to inform the diagnosis. The typical blood alcohol concentration for prenatal alcohol exposure is assessed using body weight and frequency of drinking [36]. Persistent alcohol use increases tolerance, which can result in very high blood alcohol concentrations for some individuals, without them feeling the effects of alcohol. This can make it difficult to identify the problem of drinking. Another key factor is the effect of drinking on consecutive days, when the second exposure episode begins before the blood alcohol concentration from the previous drinking episode has reached zero. In these situations, a higher blood alcohol concentration for the second episode will result leading to an extended period of exposure for the fetus. Despite many years of investigation, a safe threshold for alcohol consumption during pregnancy has not been identified [37].

Confirming Maternal Alcohol Exposure

The first step of the diagnostic process is confirming prenatal alcohol exposure. Thus, one of the most difficult issues related to FASD diagnosis is the lack of accurate antenatal records with documented prenatal alcohol exposure. Compounding the problem is the fact the majority of the individuals with FASD do not have the three identifying facial features [38, 39]. In these cases, a diagnosis is even more challenging when the brain injury is mild and the majority of the behavioural and intellectual deficits do not present until the child is school-age. Due to denial, embarrassment, and litigious fears, maternal reports of gestational drinking are often inaccurate or not available [40].

Based on the literature and clinical experience, it is evident that substantial differences exist in the screening and referral processes used for alcohol, including during pregnancy. The Society of Obstetricians and Gynaecologists of Canada recommends that all pregnant women and women of child-bearing age should be asked periodically about alcohol, tobacco, prescription drug use, and illicit drug use, and that pregnant women at-risk for problematic substance use should be offered brief interventions and referral to community resources for further psychosocial support [41]. The American College of Obstetricians and Gynecologists have similar recommendations [42]. In a review of the prenatal record forms in Canada, most lacked the appropriate prompts to encourage providers to intervene or refer pregnant patients with high-risk drinking behaviours to seek appropriate treatment [43]. Additionally, it was speculated that the level of 'at-risk' drinking during pregnancy was underestimated across Canada, as less than half of the prenatal record forms included the use of validated screening tools (i.e., T-ACE, TWEAK). Of the records that included prenatal screening tools, questions related to the amount(s) and type(s) of alcoholic drinks consumed, the pattern of drinking and the frequency were not included. Failure to identify at-risk alcohol consumption during pregnancy, or to appropriately intervene, may increase the likelihood of an alcohol-exposed fetus. Currently, the evidence-based recommendations related to the prevention and diagnosis of FASD have not been consistently integrated into prenatal record forms.

Health care providers are often uncomfortable discussing sensitive topics such as alcohol consumption with their pregnant patients and do not possess a good understanding of the effects of alcohol on the developing fetus or are unaware of the appropriate interventions once alcohol use has been confirmed [44]. Documentation and maternal history of substance use are both important components for identifying an individual with prenatal alcohol exposure, which is critical for a diagnosis in situations where there are no physical features.

For patients, who were adopted or in foster care, obtaining confirmation of prenatal alcohol exposure can be another challenge for diagnosis [45, 46]. Without a record of antenatal history, FASD diagnoses are near impossible. Documented antenatal records are difficult to obtain for many children in care; a problem that is compounded when the adoption is International [45, 47]. Risk factors such as "a history of alcoholism", "multiparous" and "age" (i.e., older at the time of an affected pregnancy) can all be used when considering an FASD diagnosis. It is imperative that nurses, physicians, and other health care providers become comfortable with obtaining a history of prenatal alcohol use and training to provide the appropriate follow-up care and guidance.

Biomarkers

Biomarkers have been under investigation to assist with obtaining confirmation of prenatal alcohol exposure. The most common clinical tool is maternal self-reporting. However, a more objective approach is the use of biomarkers from biological specimens alone or in combination with maternal self-reporting [48]. It has been reported that the biomarkers fatty acid ethyl esters, ethyl glucuronide, ethyl sulfate, and phosphatidyl ethanol may be promising indicators for the detection of prenatal alcohol exposure [48]. Other potential biomarkers include ultrasound measurements of the fetal corpus callosum [49] and deficits involving the visual system [50–54].

The discussion and development of potential biomarkers for prenatal alcohol exposure are not without significant ethical implications. Biomarkers for FASD pose several unique challenges due to the nature of the information obtained and the implications. Positive screens include a greater element of social risk for parents, particularly mothers (i.e., public exposure of their substance use, the potential for child welfare involvement, apprehensions, and legal consequences). Moreover, there are many risks related to introducing a technology before the ethical ramifications have been thoroughly considered, especially the role and rights of the parent, physician and state [55]. The key ethical issues have been summarized as follows: targeting populations for prenatal alcohol exposure screening; consent and respect for persons; stigma and participation rates; the cost-benefit of a screening program; the sensitivity, specificity and associated outcomes of a screen; confidentiality and appropriate follow-up to positive screen results; and the use of screen results for criminal prosecution [56]. Interestingly, it is the stakeholder perspectives (e.g., parents, health care providers) that have been absent in most of the reviews of the ethical issues surrounding a prenatal alcohol exposure screen. These perspectives are needed to provide appropriate insight into the potential impact and implications of biomarkers.

The Physical Examination and Differential Diagnosis

The diagnostic process should include a comprehensive social and medical history, and a complete physical examination [1]. The dysmorphology assessment is used to differentiate the specific physical features associated with prenatal alcohol exposure from those that arise due to other causes. Specific facial abnormalities have been described for the FASD population [31].

Facial Features

The revised Canadian diagnostic guidelines recommend the following criteria:

- Simultaneous presentation of 3 sentinel facial features (short palpebral fissures, smooth philtrum and thin upper lip)
  - Short palpebral fissures, at or below the third percentile (2 standard deviations below the mean).
  - Smooth or flattened philtrum, 4 or 5 on the 5-point Likert scale of the lip-philtrum guide [57, 58].
  - Thin upper lip (rank 4 or 5 on the lip-philtrum guide).
- In all cases, any signs of other congenital anomalies should be recorded, including microcephaly.

The classic triad of facial features associated with FASD includes short palpebral fissures, smooth or flattened philtrum and a thin vermilion border of the upper lip [1]. However, the presence of fewer than 3 facial features in individuals with FASD is more common, with 39% of individuals in a large Canadian database having no sentinel features, 24% having one, 15% with two and 14% with all three [39]. The presence of facial features continues to play an important role in the assessment for FASD, where prenatal also exposure cannot be confirmed, although differences in facial variation in human subjects and the reliability of alcohol intake information pose significant challenges for establishing dose-response relationships. Animal models; however, enable the study of facial alterations related to specific timing and dose of alcohol. In the mouse model of FASD, facial morphometric analysis in parallel to anthropometry was used to successfully identify pups exposed to prenatal ethanol following a specific dose and time of alcohol exposure [59]; illustrating the utility of experimental mouse models to better define risk factors (e.g., dose, timing, frequency) that contribute to the facial phenotype in FASD. The implications for the clinical situation are unknown, but in future, it may be possible to determine alcohol dosimetry from the severity of a given facial feature.

Advancements have also been made to the tools that are used to collect information about facial dysmorphology. Using a unique set of facial regions and features, 2 and 3D facial scanned images were used to develop an automated technique that could accurately discriminate subjects with FAS from controls, without human intervention [6, 60]. As well, a stereo-photogrammetric tool for the diagnosis of FAS in infants has proven highly precise and reliable [61], which is promising given that, with early intervention, the prognosis of FASD improves substantially and development of secondary disabilities decreases [62].

There are a number of studies exploring possibilities for using technology to help in the diagnosis of FASD, especially related to facial features. For example, heat maps and morphing visualizations of face signatures are being explored and may help clinicians detect facial dysmorphism across the fetal alcohol spectrum in the future. Face signature graphs show potential for identifying non-syndromal, heavily-exposed children, who lack the classic facial phenotype, but have cognitive impairments [2].

Craniofacial development is intimately linked to the development of the brain; though, the relationship between facial directional asymmetry (associated with prenatal alcohol exposure) *and* structural asymmetry of the brain remains to be determined. It is likely that a range of developmental processes, and their disruption, can lead to associations between the structure and function of the brain, and facial shape and symmetry. This continues to be investigated.

Foroud *et al* related anthropometric and cognitive measurements in children at 5 and 8 years of age with and without FASD to determine if the face predicts brain (dys) function [63]. Several anatomical features were predictive of group membership (FASD or control), such asmeasures of craniofacial width (minimal frontal), orbital width (palpebral fissure width), and ear and mandibular measures (ear length and lower facial depth). Additionally, facial width, length, and depth measurement were highly sensitive and specific for differentiating children with FASD from controls. Sensitivity and specificity were also high for discriminating children FASD from the heavily-exposed children, who did not meet criteria for FASD. These results provide further insight into the complex relationship between the face and the neuropsychological deficits that occur following prenatal exposure to alcohol and may, in the future, inform new diagnostic tools.

The Neurobehavioural Assessment

Diagnostic Criteria from the Canadian Guidelines

- Evidence of impairment in *3 or more* of the following central nervous system (CNS) domains:
  - Motor Skills
  - Neuroanatomy /Neurophysiology
  - Cognition
  - Language
  - Academic Achievement
  - Memory
  - Attention
  - Executive Function, including Impulse Control
  - Affect Regulation
  - Adaptive Behaviour, Social Skills or Social Communication

A severe impairment is defined as a global score or a major subdomain score on a standardized neurodevelopmental measure that is ≥2 standard deviations below the mean, with appropriate allowance for test error. In some domains, large discrepancies among subdomain scores may be considered when a difference of this size occurs with a very low base rate in the population (≤3% of the population). Clinical assessment with converging evidence from multiple sources and DSM-5 diagnostic criteria for certain disorders may also be considered in specific domains which are not easily assessed by standardized tests (for example, in the affect regulation domain the following diagnoses may be taken as an indication of severe impairment: Major Depressive Disorder (with recurrent episodes), Persistent Depressive Disorder, Disruptive Mood Dysregulation Disorder (DMDD), Separation Anxiety Disorder, Selective Mutism, Social Anxiety Disorder, Panic Disorder, Agoraphobia, or Generalized Anxiety Disorder).

The neurobehavioural deficits associated with FASD are multifaceted and vary from mild to severe depending on the situation. In particular, executive functions (those requiring higher levels of cognitive processing) and working memory are typically considered hallmark features of FASD [64–66]. Mental health comorbidities are prevalent among individuals with FASD, and they are also at risk for suicide and addiction [67, 68]. Importantly, both the executive function deficits and mental health issues can occur in the absence of facial dysmorphology, making the neurobehavioural assessment critical to the diagnostic process.

FASD and Mental Health

A disproportionate number of individuals with FASD have mental health comorbidities [5, 67, 69]. These problems are often present in childhood and adolescence and persist into adulthood, where mental health problems are considered the most severe characteristics of FASD [70]. The most prevalent mental health issues for individuals with FASD are depression, mood and anxiety disorders, attention-deficit/hyperactivity disorder (ADHD) and conduct disorder (CD). For a comprehensive review of FASD and mental health, please refer to Easey et al. [67] and Pei et al. [68].) Individuals with FASD are also at risk for high rates of suicide [71] and addiction [72]. A systematic review revealed that there were 15 comorbid mental health disorders associated with FASD; ADHD occurred in 50% of all FASD cases (10 times the expected rate) and intellectual disability occurred at 23 times the expected rate. In 5 of the 12 comorbidities, rates in the FASD population significantly exceeded expected rates by 10% to 45% [73].

Others have also shown that ADHD is one of the most common comorbidities in patients with FASD [68, 74, 75]. Several human studies have contrasted cognitive abilities

using standardized psychometric tests to characterize the differences in performance between the two clinical populations. Interestingly, although both groups demonstrate neurodevelopmental deficits, the nature and/or mechanisms for the deficits differ, as revealed on tests of spatial working memory [76], adaptive behaviour [77, 78], verbal learning and memory [79], executive function [80] and arithmetic [81]. These data suggest a unique mechanism for the effects of prenatal alcohol exposure on specific neurodevelopmental outcomes.

In a study of children and adolescents (age 8–16 years), with and without prenatal alcohol exposure and ADHD, an elevated risk of psychiatric disorders and behavioural problems were associated with both ADHD and prenatal alcohol, compared to controls [82]. Findings revealed that an ADHD diagnosis elevated the children's risk of psychiatric diagnoses, regardless of prenatal alcohol exposure. However, co-occurrence of ADHD and prenatal alcohol exposure exacerbated the occurrence of conduct disorders and externalizing behaviours. Furthermore, there was a poor behavioural prognosis for alcohol-exposed children with ADHD, suggesting the potential for more than one neurobehavioural profile for individuals with FASD [82]. These observations are important for mental health care providers, who should routinely consider the possible contribution of prenatal alcohol exposure in the diagnosis and management of mental illness and developmental disorders. Because of the potential neurological (functional and structural) effects resulting from prenatal alcohol exposure, unique screening and intervention approaches may be required for individuals with FASD.

Imaging as a Diagnostic Tool

Imaging and brain metabolism techniques provide unique opportunities to evaluate brain function *in vivo*. Several excellent reviews describe and summarize the major advancements in understanding the relationship between neural activation and the behavioural outcomes associated with FASD [83, 84]. Data reveal that there are specific differences between those with FASD and those with other disorders, including ADHD [83]. Morphological differences in the brain, and how these differences relate to cognitive deficits and facial dysmorphology, have also been described. New technologies have provided valuable insight into the relationship between white matter microstructure and behaviour, atypical neuromaturation across childhood and adolescence, and differences in neural activation patterns underlying sensory processing, cognition and behavioural deficits associated with FASD, including reductions in global network efficiency [84]. Microcephaly and disproportionate reductions in the size of the parietal lobe, cerebellar vermis, corpus callosum and caudate nucleus, have all been associated with prenatal alcohol exposure [85, 86]. Despite these advancements, there are still many unknowns about the impact of prenatal alcohol exposure on the brain, and continued research efforts are essential to understanding the complex mechanisms underlying the resultant neurodevelopmental deficits.

Treatment and Follow-Up

Clinically, a major problem for FASD diagnosticians is follow-up. Depending on where patients obtain their diagnosis will often dictate the type of follow-up services and supports they will receive. In some cases, the diagnosticians do not know where to refer their patients after a confirmed diagnosis is made, making management extremely complex. To further complicate the issue, many individuals with FASD also present with significant comorbidities that must be considered in devising appropriate treatment strategies. Comorbidities substantially increase the difficulty of management over time; underscoring the need for longitudinal assessments from infancy to adulthood [75]. A primary goal when working with the FASD population is to provide anticipatory guidance about future developmental abnormalities and to provide appropriate therapeutic interventions when they present and when feasible. A 10-year management plan has been implemented by some clinics to provide individuals with the opportunity to review their current situation and anticipate upcoming problems. The three common goals to management include (1) prevent multiple foster home placements (i.e., the uprooting and re-assimilation), which are extremely detrimental for optimal development; (2) maximize parent or caretaker understanding of the age-related changes in behaviour and age-related risks; and (3) anticipate future development of age-related impairments common in FASD [75].

Individuals with FASD can present with a wide variety of complex mental health and behavioural problems that require a multifaceted approach to treatment and management. The heterogeneity of the FASD population is further impacted by differences in additional pre- and post-natal insults and adverse events. Unfortunately, with respect to treatment, the demand far exceeds the supply, and many patients are unable to obtain the appropriate services and support due to limitations in availability and accessibility.

The complexity and persistence of FASD-associated problems require a long-term plan for management, especially as the individual matures. Eleven primary intervention categories and subcategories were identified in a retrospective analysis to identify the types and frequencies of sup-

ports, services, and referrals recommended by an interdisciplinary diagnostic team for children and youths with FASD [87]. Educational and medical needs were the most common recommendations for individuals with FASD. Comprehensive psychoeducational or neuropsychological assessments; special education programs, services, or eligibility; and advocacy to enhance or modify existing educational programs or services were the most frequent recommendations in the education category. For medical referrals, psychiatric care and/or medication management; vision and/or hearing screening; and neurological consultation or treatment were most common. Interestingly, the recommendations were relatively comparable across diagnostic sub-groups; though differences did emerge when stratified by age. Children in the birth-2 year cohort received more recommendations for family support and social service-child welfare interventions, while for children in the older group, recommendations were predominantly for mental health services and community resources.

Community home-based attachment interventions, such as Circle of Security® (COS), have been beneficial for preschool children affected by FASD [88]. Recent data from Canada's National FASD Database report that a large breadth of supports and services are recommended to individuals and families as part of the diagnostic process [39]. Zarnegar et al. suggest that using neurodevelopmentally-informed assessment strategies to sequence interventions for young children with diverse neurodevelopmental insults is a promising intervention approach that improves outcomes [89].

Specific Populations

FASD Diagnosis in Infants

For many reasons, diagnosis of FASD at the time of birth is a major challenge; however, there is potential to provide an assessment to newborns, who are at-risk, during infancy (0–18 months). Several interesting studies have evaluated infants, who were affected by prenatal alcohol exposure, to identify the emerging neuropsychological profile and specific risk factors that may be assessed during early physical examinations. Prenatal alcohol exposure has been associated with infant temperament problems [90], difficultness [91], problems with crying, sleeping and eating [92], blunted pain responses [93], poor visual acuity [94], general developmental delays [95], and in one case report, severe gastroesophageal reflux [96]. Prenatal alcohol exposure has also been associated with smaller corpus callosum in newborn MRI scans [97]. Infant symbolic play (i.e., ability of children to use objects, actions or ideas to represent other objects, actions, or ideas as play) has also been evaluated as a potential predictor of FASD [98], though more research is required to identify additional diagnostic tests that can be effective in infants and small children.

FASD Diagnosis in Adults

Based on the available epidemiological data, it is suspected that there is a large population of adults with FASD; however, there are significant limitations in the availability of diagnostic and treatment services. For adults, the impairments predominantly involve social adaptive skills and executive functions, and there is a high degree of psychopathology. These situations represent the cases where a diagnosis has been obtained; though, it is suspected that many adults do not receive the appropriate medical diagnosis and subsequent treatment. These latter cases are enigmatic. With the development and implementation of better screening and diagnostic tools geared towards adults, a more accurate indication of the situation and the specific needs for adults with FASD will emerge. [Please see the following review for a description of the challenges associated with diagnosing FASD in adults [99]].

To date, a large proportion of the information pertaining to FASD in the adult population and the mechanisms by which prenatal alcohol exposure leads to dysfunction (neurological, behavioural and physical) have been obtained from the animal literature. For example, the long-term effects of neonatal alcohol exposure have been associated with impairments in circadian rhythm [100], impairments in sleep-wake behaviour [101] and auditory brainstem response abnormalities (i.e., the indication of hearing and neurological function) [102] in animal models of FASD. As well, prenatal exposure to alcohol may alter responsiveness to stress in the adult offspring [103]. In human studies, structural and physical abnormalities associated with prenatal alcohol exposure include cardiovascular arterial stiffness [104] and reproductive issues [105], including male infertility [106].

Several imaging studies have also revealed insight into the adult FASD brain. Using diffusion tensor imaging—which provides a visual representation of the connections in the brain—changes in white matter integrity of the corpus callosum was revealed in young adults with FASD compared to controls [107]. In future, this could serve as a potential marker for prenatal alcohol exposure. Brain metabolic alterations have also been revealed using magnetic resonance spectroscopy in adults with FASD [108, 109].

Studies describing the adult FASD phenotype reveal that the deficits associated with prenatal alcohol exposure are persistent and debilitating [see review [110]]. Social deficits persist across the lifespan and may worsen with age, inde-

pendent of IQ and dysmorphology [111] and occupational difficulties that are characterized by disruption, failure and severe social problems [112].

Conclusion

In conclusion, diagnosing any individual with FASD continues to be a major clinical challenge that is compounded by a lack of resources and inconsistencies in diagnostic procedures and follow-up care. Management and treatment plans vary dramatically based on the clinic, the services available and the diagnostic approaches used. Despite this, there have been significant achievements in the efforts to move FASD to the forefront of public awareness and policy campaigns. Research is ongoing and continues to reveal novel discoveries that will help improve the technologies that are available for screening, diagnostics and treatment. This is an exciting time for FASD innovation, with the anticipated evolution of evidence-based, international guidelines and definitions. This will not only improve the understanding of FASD diagnoses but also define prevalence around the world.

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Multiple Choice Questions

1. Which of the following nomenclature for FASD is not a diagnosis?
   a. FASD with Sentinel Facial Features
   b. FASD without Sentinel Facial Features
   c. **At Risk for Neurodevelopmental Disorder and FASD, Associated with Prenatal Alcohol Exposure**
   d. Alcohol-Related Neurodevelopmental Disorder
2. Which of the following diagnostic criteria is no longer needed for diagnosis?
   a. **Growth**
   b. Facial dysmorphology
   c. Brain dysfunction
   d. Congenital abnormalities
3. How is neurodevelopmental impairment calculated?
   a. < 1 standard deviation below the mean
   b. < 1.5 standard deviations below the mean
   c. **< 2 standard deviations below the mean**
   d. < 3 standard deviations below the mean
4. What is the minimum number of domains of impairment required to confirm neurodevelopmental deficits for a FASD diagnosis?
   a. 1 domain
   b. 2 domains
   c. **3 domains**
   d. 5 domains

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Magnetic Resonance Imaging in Fetal Alcohol Spectrum Disorder (FASD)

25

Catherine Lebel and Ashley Ware

Learning Objectives

- To understand the structural and functional brain alterations in FASD across the lifespan
- To know the most common neuroimaging findings associated with FASD
- To understand that alterations in structural and functional brain connectivity occur with FASD, but may not be evident on standard MRI
- To recognize that brain alterations associated with FASD are linked to cognitive difficulties and behavioural problems

Highlights

- Gross abnormalities on MRI are uncommon in FASD, though more subtle alterations are common
- Smaller brain volumes are the most consistent finding, and have been reported in infants, children, adolescents, and adults
- Structural and functional brain alterations are linked to cognitive difficulties and behaviour problems
- More advanced imaging techniques and longitudinal studies offer great promise for important discoveries in the coming years

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Introduction

Over the last 25 years, magnetic resonance imaging (MRI) has significantly advanced our understanding of brain alterations associated with prenatal alcohol exposure (PAE) by allowing non-invasive, in vivo measurements of the brain. The use of advanced MRI in large, longitudinal studies of individuals with PAE has provided far more detail about the largely subtle and heterogeneous brain alterations that occur following PAE than initially identified by post-mortem autopsy [1–7]. This chapter will highlight some of the key findings of brain alterations in individuals with FASD from MRI studies.

Fetal alcohol spectrum disorder (FASD) is a neurodevelopmental disorder that is caused by PAE and characterized by life-long cognitive, behavioural, and neurological deficits [8]. The estimated prevalence of FASD in North America is 2–5%, but higher in certain regions of the world [9, 10]. FASD represents a significant economic burden, with lifetime costs estimated at over $1,000,000 per individual [10–12]. A diagnosis of FASD typically requires confirmation of PAE and cognitive, behavioural, and/or emotional dysfunction; specific diagnostic criteria are reviewed in the previous chapter (see Diagnosis and Assessment). Beyond the primary cognitive and behavioural deficits, over 90% of individuals with FASD experience co-occurring mental health problems [13–16]. This likely reflects the fact that PAE can cause both structural and functional brain alterations across the lifespan.

Structural brain alterations following PAE were initially characterized using post-mortem autopsy [1–7]. These studies showed marked microencephaly and malformations of the corpus callosum, including partial and complete agenesis. Cortical and subcortical alterations were also observed. It is noteworthy that these studies typically only captured the effects of PAE at the most severe end of the FASD spectrum since early infancy and childhood death rarely occurs except in the most severely affected cases. The advancement of clinical MRI has enabled a much broader range of individuals

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with PAE and FASD to be studied. MRI is a non-invasive neuroimaging technique that can safely be used in cross-sectional and longitudinal research to study *in vivo* brain structure and function. While MRI is not the only neuroimaging tool that has been used in research on FASD, it has been the most extensively utilized tool *by far* [17, 18], and is the focus of the current chapter. Rather than an exhaustive review of MRI studies in FASD, this chapter will provide a summary of key findings that have emerged over the last 25 years, following a brief introduction to the most commonly used MRI techniques in studies of FASD. This review also highlights select studies that have been paramount in understanding specific brain differences resulting from PAE as well as important links between brain alterations and neuropsychological (i.e., cognitive and behavioral) outcomes. Since most research on FASD to date has examined children and/or adolescents, that remains the focus of this chapter, although findings in adults with FASD are included where relevant. A detailed review of the literature on adults with FASD is available elsewhere (e.g., [19]).

MRI offers numerous contrast mechanisms to investigate brain structure and function; the most common types of MRI that have been used to study FASD are T1- or T2-weighted anatomical imaging, diffusion tensor imaging (DTI), and functional MRI (fMRI). Anatomical (structural) imaging provides excellent contrast of brain structure as a result of differing tissue densities and relaxation properties. This tissue contrast can be used to extract quantitative indices of total brain volume as well as the volume, thickness, and surface area of distinct cortical and subcortical gray and white matter regions within the brain. This is often accomplished using automated segmentation programs like FreeSurfer [20] and MACRUISE [21]. DTI assesses the microstructural properties of subcortical white matter connections via measures that capture aspects of constricted water diffusion. Fractional anisotropy (FA) and mean diffusivity (MD) are the most commonly used DTI metrics. FA is a measure of the directionality of diffusion; it is sensitive to myelination, axonal density, and axon coherence [22]. MD is a measure of the overall water movement occurring within the brain. Functional MRI is based on hemodynamic response and can identify brain regions that are active during particular tasks (task-based fMRI). Resting-state fMRI (rs-fMRI) measures patterns of spontaneous brain connectivity based on correlated functional signals among isolated brain regions to determine which regions fire in parallel or sequentially and are thus functionally connected (i.e., degree of "functional connectivity") [23].

Radiological findings in FASD. Visually apparent brain alterations that could easily be diagnosed by a radiologist have been observed in many individuals with PAE. Early neuroimaging studies of FASD revealed substantial brain abnormalities on clinical MRI scans. This includes microcephaly, partial and complete agenesis of the corpus callosum, and cerebellar hypoplasia [24–26]. Those early studies only examined a limited number of children. High rates of brain abnormalities were more recently reported in larger samples, which indicated that 62% [27] or 84% [28] of cases exhibited clinically significant neuroradiological alterations. For instance, Fig. 25.1 shows a range of alterations in the corpus callosum reported in individuals with PAE [28]. While informative for understanding structural brain alterations caused by PAE, these rates do not necessarily reflect the broader PAE population and could even be inflated given that the included sample of children had histories of very heavy PAE and/or significant cognitive or behavioural deficits that warranted clinical investigation. Systematic examinations of brain alterations in children and adults with PAE who were scanned for research instead of clinical purposes further indicate that neuroradiological abnormalities may indeed be less common in the general population of individuals with PAE, who represent a less severely affected population of individuals with PAE, than in those who complete clinical neuroimaging [29].

When reviewed by a neuroradiologist who is blinded to PAE history, individuals with PAE do not show significantly greater rates of incidental findings compared to unexposed, healthy controls [29]. For instance, children and adults with PAE only showed a similar number of total incidental (27% vs. 25%) and clinically significant incidental (5% vs. 3%) findings compared to controls, with the most common incidental finding being ventricular enlargement [29]. However, children with alcohol-related facial dysmorphology are significantly more likely to have at least one incidental (41% vs 25%) and clinically significant incidental findings (8% vs 1%) than controls. More specifically, 12.5% of children with more severe FASD had encephalopathy and 15% of children with fetal alcohol syndrome or partial fetal alcohol syndrome had a clinical abnormality such as Chiari malformations and alterations of the corpus callosum [29]. These overall results support a dose-dependent effect of exposure history, with heavier PAE associated with greater brain alterations.

In sum, gross brain alterations including malformation of the corpus callosum and cerebellum, and enlarged ventricles are more common in individuals with heavy PAE than healthy controls and are readily apparent on MRI. However, these neurological abnormalities are not typical of individuals with PAE. Instead, more heterogeneous populations of participants with PAE reveal similar rates of incidental findings to those seen in unexposed, healthy controls, suggesting that major brain alterations are most common with heavier exposure histories and are not present in most individuals with PAE or FASD.

In contrast to qualitative neuroradiological ratings, quantitative MRI methods can reveal more subtle brain alterations in individuals with FASD and have been used extensively

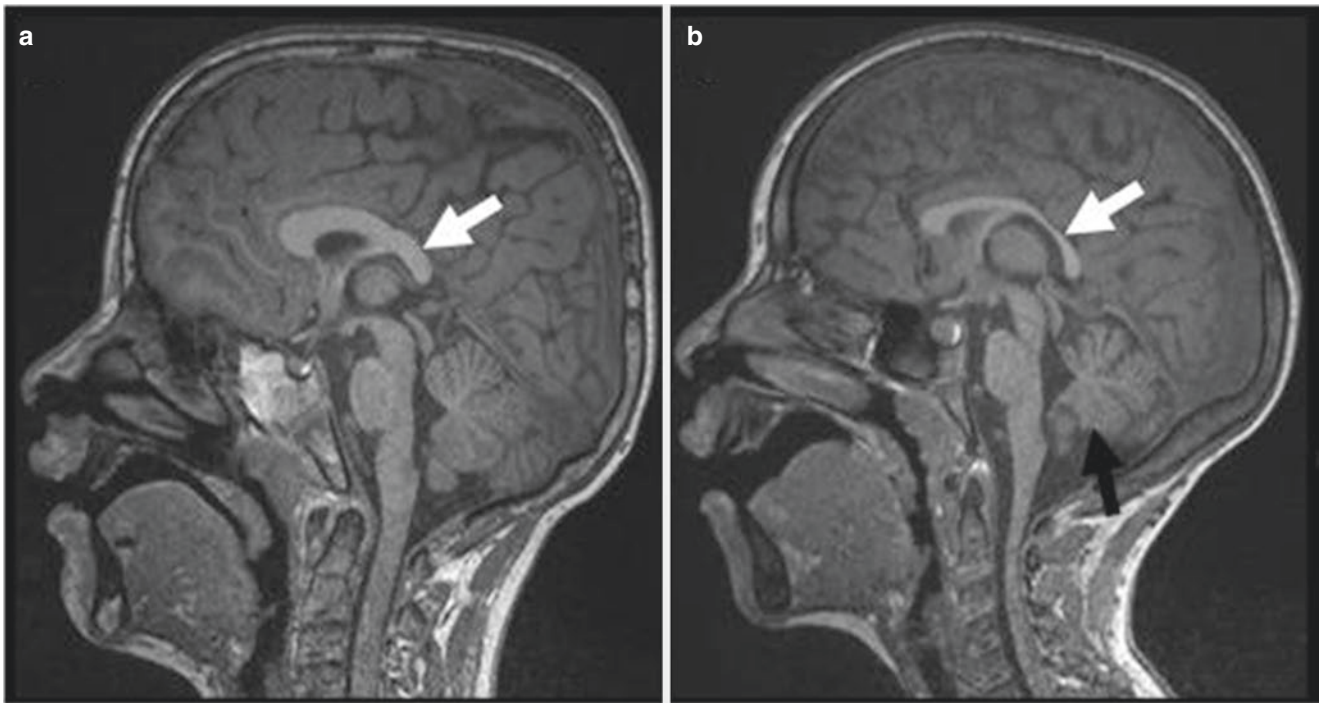

Fig. 25.1 Structural brain alterations seen in patients with prenatal alcohol exposure. (**a**) and (**b**) show malformations of the corpus callosum (short and thin, respectively). (**b**) also shows hypoplastic cerebellar vermis (black arrow). Figure adapted from [28]

over the last three decades. While much remains to be discovered about brain alterations associated with PAE, some consistent findings are emerging.

Quantitative MRI findings in FASD

A growing body of evidence-based on quantitative structural MRI emphasizes the largely subtle nature of structural brain abnormalities that exist across children and adolescents with PAE. We note that more comprehensive reviews of micro- [30–32] and macro-structural [18, 33] alterations in PAE have been published elsewhere.

Brain structure in FASD

The most common structural brain alteration reported in individuals with FASD in quantitative MRI studies is reduced brain volume [18, 30]. Total brain volume and volume of brain substructures are consistently reduced in children, youth, and adults with PAE. Earlier studies suggested that frontal-parietal areas, particularly the white matter, were most substantially reduced in volume than other areas [34, 35], although findings have not been entirely consistent in terms of regional differences [18, 30].

Beyond gross abnormalities, research studies have used numerous methods to detect subtle brain alterations in children and adults with FASD, including analyses of white matter microstructure, thickness, and shape. The corpus callosum is a midline structure known to be vulnerable to the teratogenic effects of alcohol and is among the most consistently reported structures. This region has consistently demonstrated smaller volume [35–37], altered shape [38–40], and weaker connectivity [41–44] in individuals with PAE. However, greater evidence for alterations may partially reflect the fact that the corpus callosum is a large structure that has more often been investigated, rather than it being particularly uniquely vulnerable.

Macro- and microstructural aberrations also exist in frontal and subcortical gray and white matter [18, 32]. The caudate and putamen, which together form the striatum, are disproportionately reduced in children with PAE once total brain volume reductions are taken into account [45–47]. Other deep gray matter structures also are substantially altered in children with prenatal alcohol exposure, demonstrating smaller volumes [35, 36, 47, 48], altered microstructure [41], and shape differences [49]. The hippocampus, for instance, may be especially sensitive to the teratogenic effects PAE [50, 51]. Finally, cerebellar malformations have been demonstrated in children and young adults with FASD [24, 52, 53].

In children with FASD, studies have reported both thicker [54–56] and thinner cortex [57, 58] compared to unexposed controls. Software packages vary in how they calculate cortical thickness and may lead to slight differences in findings;

however, processing differences should not produce completely opposite results. Instead, these seemingly conflicting findings may be explained, at least in part, by altered trajectories of brain development. Longitudinal neuroimaging studies show altered cortical development trajectories during childhood and adolescence, suggesting that thickness differences between individuals with and without PAE may change over time [59–61]. Specifically, individuals with PAE have slower changes in cortical thickness and volume, suggesting reduced plasticity and leading to thinner cortex in early childhood and later adolescence, but thicker cortex during middle childhood. Thus, differences in the age range and mean age of participants across studies may contribute to apparently conflicting findings. Further longitudinal studies are necessary to better understand the regional and temporal variation of differences in cortical thickness.

White matter microstructure has been most often measured in individuals with FASD using diffusion tensor imaging (DTI) metrics, which most commonly include fractional anisotropy (FA) and mean diffusivity (MD). Children and youth with PAE consistently show lower FA and higher MD, reflective of weaker microstructural connectivity and often interpreted as lower white matter "integrity". The corpus callosum, as mentioned above, has been highlighted as frequently showing abnormalities [41–44]. Broader white matter connectivity alterations have also been observed, using a variety of data analysis methods, in white matter tracts in all parts of the brain [32, 41, 42]. More recent research suggests that opposing patterns may be observed in infants and younger children with PAE, namely higher FA and/or lower diffusivity than unexposed controls [62, 63]. This may reflect atypical brain maturation.

Brain Function in FASD

Altered brain function can be assumed by the cognitive and behavioural problems commonly associated with FASD [64, 65], but advanced MRI techniques have provided more direct insight into which parts of the brain demonstrate atypical activation, blood flow, and/or connectivity and lead to cognitive and behavioral dysfunction.

Two general themes emerge in functional brain imaging of individuals with PAE. The first is that of inefficient brain activity underlying performance during cognitive tasks that tap typical neuropsychological weaknesses in individuals with PAE. The second theme supports functional compensation through abnormal recruitment of brain regions in this population as compared to unexposed controls. Discrete cognitive domains have been investigated using fMRI in youth and, to a much lesser extent, adults with FASD, including learning and memory as well as arithmetic [66, 67]. Altered brain function is also seen when children and adolescents are at rest [68]. However, cognitive components of executive control have most extensively been examined using task-based fMRI in children and adolescents with PAE, particularly inhibitory control and working memory [69–75].

The frontal-striatal network, which is comprised of neural circuits among regions within the prefrontal cortex, striatum, and thalamus, and involved in goal-directed behaviors like inhibitory control and behavioral regulation, shows particular vulnerability to the effects of PAE [72]. Collectively, the published literature suggests that frontal-subcortical networks function inefficiently in this population relative to controls [70, 72, 76]. Children and adolescents with PAE also recruit different brain regions than controls during working memory and response inhibition tasks [70, 72, 73, 76]. In a study of response inhibition, adolescents with PAE demonstrated greater recruitment of several of the brain regions that are implicated in successful response inhibition relative to unexposed controls during similar performance accuracy as trial difficulty increased [72]. Specifically, the left middle and dorsal anterior cingulate, thalamus, and caudate were recruited to a greater extent. Increased recruitment and altered functioning of prefrontal regions support disrupted prefrontal inefficient neural functioning resulting from poor efficiency in youth with PAE. Inefficient brain function and abnormal neural recruitment during verbal and spatial working memory are supported in children and adolescents with PAE, who demonstrated greater signal in prefontal regions, and altered frontal-parietal network synchrony and coordination [71, 73].

Brain Development in FASD

Longitudinal studies not only show brain alterations but also reveal how the brain's developmental trajectories may be altered in children with FASD. Although FASD is primarily a prenatal brain injury of sorts, it results in life-long effects. Importantly, individuals with FASD do not simply show delayed or protracted development, but appear to attain key milestones through different trajectories and exhibit different sensitive periods relative to typically developing peers. This is exemplified in longitudinal studies of gray matter, which have shown more linear declines of cortical gray matter volume with age in children with PAE, suggesting earlier peak volumes than in controls [60], and less cortical thinning in children and adolescents with PAE compared to unexposed controls [61]; see Fig. 25.2. Results are more mixed for subcortical gray matter structures, with one study reporting widespread volume reductions, but no age-group interac-

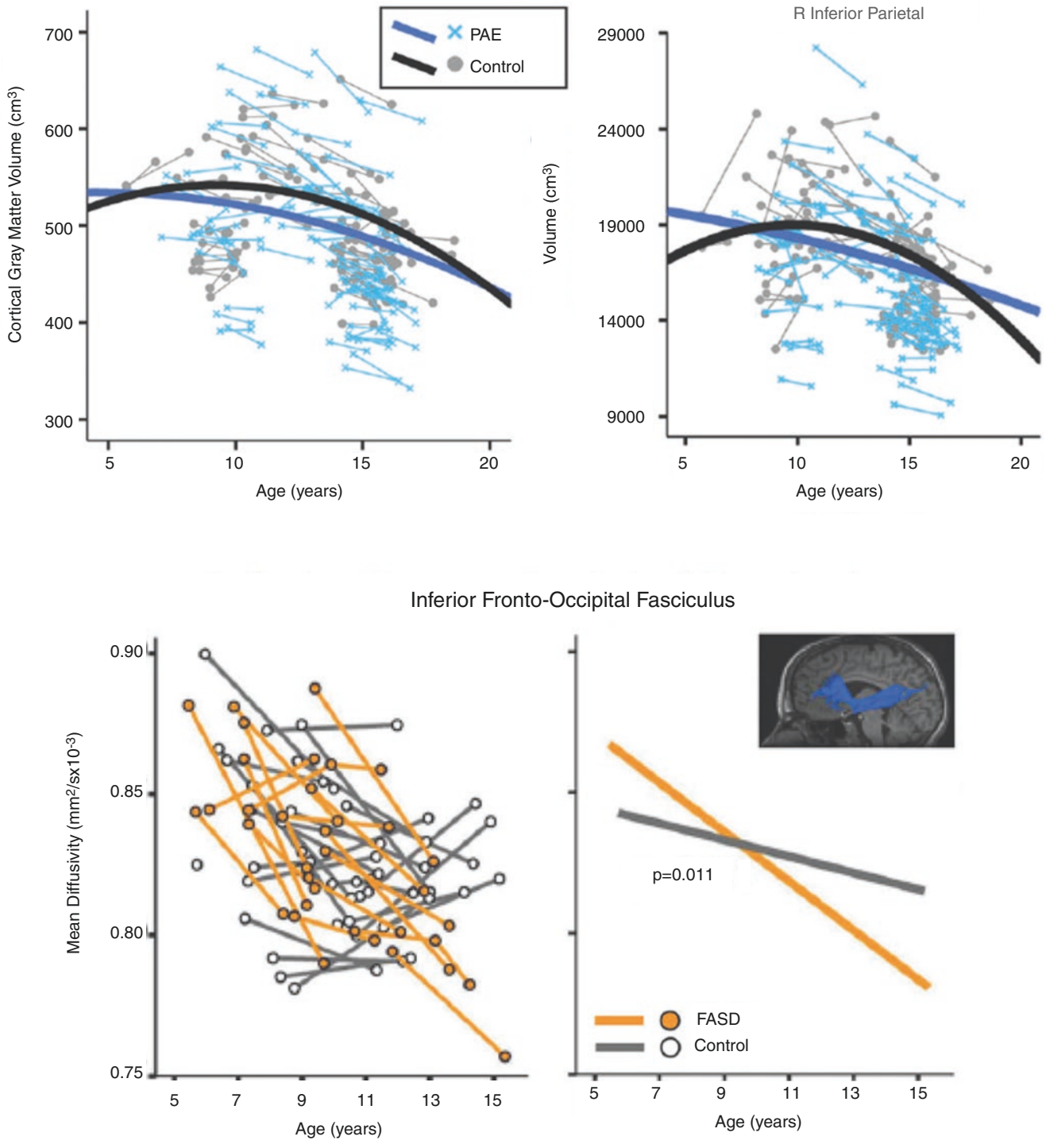

Fig. 25.2 Developmental changes in individuals with PAE are shown in gray matter volume (top) and white matter microstructure (bottom). Interestingly, individuals with PAE tend to show less overall change in gray matter, but more overall change in white matter microstructure across this age range. Figure adapted from [60, 77]

tions [78], while another reported altered trajectories in the caudate specifically [79]. Earlier peaks and more linear trajectories may reflect altered developmental pruning and reduced brain plasticity ("hard-wiring"), which could underlie the learning and behavioural difficulties observed in children with PAE. Together, this suggests that the window of maximal plasticity in children with PAE may occur at younger ages and/or end earlier than in typically developing children. This highlights the need for early recognition and intervention to optimally support these children.

Unlike gray matter, white matter changes appear to be greater during adolescence in PAE compared to controls, both in terms of white matter volume and microstructure (as measured by diffusion imaging; see Fig. 25.2) [77, 78]. It may be that this represents a catch-up phase of development, given that white matter shows marked volume reductions and microstructural alterations in late childhood [30, 41]. Although not known, it is possible that puberty could influence the neurodevelopmental trajectories in this population.

Newer Techniques

With substantial hardware and software improvements over the last few years, neuroimaging methods have become faster and more robust to participant motion. This both makes imaging more feasible in children with PAE and permits more sophisticated analyses of brain alterations in affected children. Consistent findings of reduced white matter connectivity from diffusion tensor imaging suggest that myelin may be altered in PAE. However, DTI parameters are also sensitive to axonal packing and fibre coherence [22], and thus cannot inform specific conclusions about underlying changes. A recent study used myelin water imaging to query myelin content in the brains of individuals with PAE, finding largely similar brain profiles between individuals with and without PAE [80]. Thus, it may be that white matter differences are primarily due to reduced axonal packing and/or coherence, rather than reduced myelin. Other advanced techniques, such as neurite orientation dispersion and density imaging (NODDI) [81] and inhomogeneous magnetization transfer [82] offer additional specificity to white matter properties but have not yet been applied in PAE.

Graph theory is a new technique that allows for the analysis of brain networks, rather than individual regions or connections. Graph theory is a mathematical technique that constructs a network of nodes and edges, termed a "connectome". Typically, a brain parcellation is used to define distinct brain regions as cortical nodes, and then connectivity between each pair of nodes is defined as the edges. This can be done for both functional neuroimaging data (where edges are defined by functional connectivity between nodes), and diffusion imaging data (where edges are defined by white matter connections between them). The resulting functional or structural connectomes can be analyzed in different ways by extracting global and local metrics such as efficiency, clustering coefficient, and path length that provide assessments of network connectivity at a large scale [83].

While numerous studies have used graph theory to investigate the brain's connectome in other disorders, very few have investigated PAE. The first connectome study in PAE showed reduced global efficiency and longer path length in youth with PAE compared to unexposed controls [84]. Two other studies have failed to show mean group differences in the functional connectome in PAE. However, one found that the PAE group was more likely to have atypical metrics than the controls [85]. Another showed similar mean values across groups, but atypical age-related changes in connectome metrics in the PAE group, which suggest less brain variability over time, perhaps a marker of reduced cognitive flexibility [86]. A study of the structural connectome in children with PAE also showed lower global efficiency, as well as reduced intra- and inter-network connectivity in children with PAE [87]; see Fig. 25.3. Notably, the somatomotor network had reduced connectivity within itself and with most other networks, suggesting it may be among the most compromised in individuals with PAE.

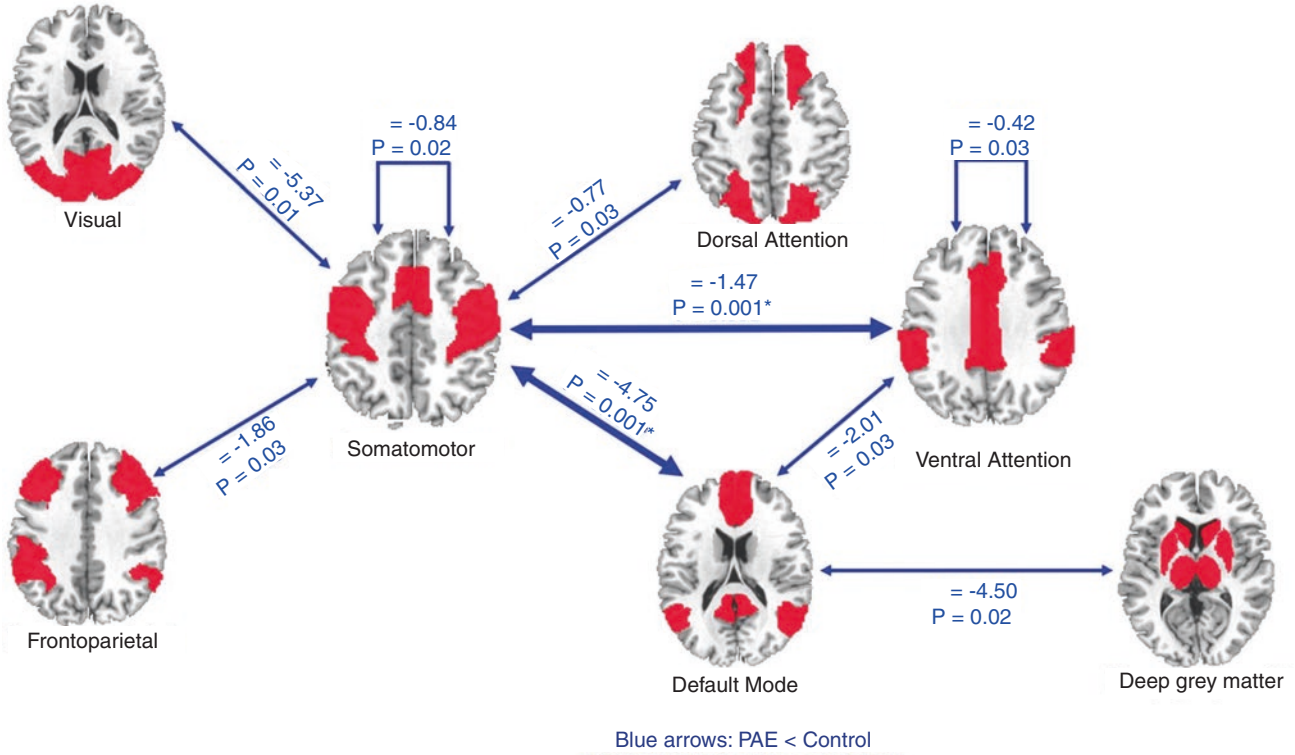

Fig. 25.3 Many key brain networks show reduced connectivity within and among each other in children and youth with prenatal alcohol exposure (PAE) compared to controls. blue lines indicate reduced connectivity either within the network itself or with other networks; the bolded blue lines survived false discovery rate correction for multiple comparisons. From [87]

Links to Cognition and Behaviour

The study of brain alterations in individuals with PAE is of particular interest because it may help advance both clinical and scientific understanding of the neuropsychological profile of affected individuals. Previous studies have related cognitive abilities and clinical features (e.g., dysmorphology) to brain structure in FASD [60, 78, 88–91]; however, it is worth mentioning that not all studies have found significant associations among brain structure and cognitive performance (e.g., [48, 75]). Associations between corpus callosum displacement, surface area, and thickness with verbal learning, Full-Scale IQ, executive function, and motor deficits have been shown in children and adults with PAE [92–94], which collectively supports the idea of reduced interhemispheric transfer efficiency. There also is ample evidence that altered frontal–subcortical structure and function in PAE corresponds with executive function difficulties in children. Reductions in deep gray matter structures, including the caudate and putamen, are associated with intellectual dysfunction in exposed children [45, 48]. Finally, alterations of the hippocampus and surrounding structures correspond with delayed recall of both verbal and nonverbal information [50, 51]. Very little is known about brain correlates of common psychiatric comorbidities, such as anxiety and depression, in FASD. Research examining such associations is warranted given budding evidence that the pituitary gland, which is implicated in some of the behavioral and mood problems commonly exhibited in children and adolescents with FASD, may be altered in this population [95].

Future Directions

New computational neuroimaging techniques have the potential to reveal more specific brain changes in children, youth, and adults with PAE. Furthermore, faster and/or quieter imaging techniques are likely to enable studies of younger children and more severely affected individuals (i.e., with more behavioural problems). A handful of longitudinal imaging studies are starting to show developmental differences in PAE, showing that effects are long-lasting and that injuries do not just persist over time, but alter how the brain matures. However, more longitudinal studies that cover wider age ranges and provide more scans per individual are needed to properly understand trajectories of structural and functional brain development in PAE, and especially to highlight when interventions may be most appropriate for behavioural and cognitive difficulties.

We are starting to gain a better understanding of the brain alterations underlying cognitive deficits in individuals with PAE, but little is known about how brain differences are related to mental health difficulties, which are experienced by over 90% of individuals with FASD [15]. Future studies expanding our understanding of brain-behaviour relationships may help better target interventions and treatments to reduce problematic behaviours and improve quality of life.

To date, most studies have focused on children aged ~8–16 years. While this is a critical period of change, as can be seen from the longitudinal studies, brain structure and function change substantially before and after this age range as well. Thus, patterns of abnormalities and brain-behaviour links observed in these children and adolescents cannot necessarily be assumed to reflect those that would be seen in younger children or adults. There are a handful of studies of infants and young children with PAE (e.g., [62, 63]), which have provided insight into the brain abnormalities. However, more studies, especially with larger samples, are needed to better elucidate brain alterations at this age. Early childhood is a critical time when the cognitive and behavioural symptoms associated with FASD typically first emerge. Studies are greatly needed to understand how the growing brain underlies these emerging cognitive and behavioural difficulties and to properly target treatments at the right time.

Conclusions

The past 25 years have seen huge gains in our understanding of the neural correlates of PAE/FASD. It is clear that gross abnormalities are the exception rather than the rule, and instead, more subtle alterations in brain structure and function are apparent. Brain volume is reduced in children with PAE, and the volume of white matter and subcortical gray matter may be disproportionately affected. White matter connectivity is weaker in children with PAE, and functional brain alterations tend to reflect inefficiencies in brain processing. These structural and functional alterations have been linked to cognitive performance and behaviour, including inhibition, working memory, and mathematics. Newer imaging acquisition and analysis techniques that have emerged over the last few years promise more specificity in imaging findings and will further elucidate the specific brain differences in FASD. Longitudinal neuroimaging studies are necessary to properly understand the altered brain development trajectories in FASD, and studies in young children may be particularly valuable. The next 25 years of neuroimaging in FASD offer much promise for furthering our understanding of the brain correlates of cognition and behaviour in PAE, and for targeting treatments to optimize children's potential.

Multiple Choice Questions

1. Brain alterations in fetal alcohol spectrum disorders (FASD) has been most extensively examined using which technique:
   a. *Magnetic resonance imaging (MRI)*
   b. Electroencephalogram (EEG)
   c. Magnetoecephalogram
   d. Post mortem autopsy
2. Fetal alcohol spectrum disorders (FASD) are characterized by lifelong deficits that include _____ domains.
   a. Cognitive
   b. Behavioral
   c. Neurological
   d. *All of the above*
3. Brain abnormalities reported on clinical radiological MRI scans in children with fetal alcohol spectrum disorders (FASD) commonly include:
   a. Microcephaly, partial agenesis of the corpus callosum only, and cerebellar hypoplasia
   b. Microcephaly, complete agenesis of the corpus callosum only, and cerebellar hypoplasia
   c. *Microcephaly, partial and complete agenesis of the corpus callosum, and cerebellar hypoplasia*
   d. Microcephaly and cerebellar hypoplasia
4. Fetal alcohol spectrum disorders (FASD) is associated with gray matter alterations, particularly in the:
   a. Cortex
   b. Striatum (caudate and putamen)
   c. Cerebellum
   d. *All of the above*

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Neuropathological Changes in Humans with History of Prenatal Alcohol Exposure or Diagnosis of Fetal Alcohol Spectrum Disorder

26

Marc R. Del Bigio

Learning Objectives

- Understanding FASD as a consequence of multiple drugs exposure and predisposition factors.
- How the knowledge about the Neuropathology of FASD is being developed so far.
- Recognize the unspecific nature of the morphologic findings in PNAE and FASD and why they are unspecific.

Highlights

- Prenatal exposure to alcohol, tobacco and other drugs and its related Neuropathology.
- Associated neurologic morphologic changes, global and by region.
- Morphologic changes in animal models of FASD and its limitations.
- The relationship between PNAE, FASD and preterm labor and sudden infant death syndrome.

Introduction

The term 'prenatal alcohol exposure' (PNAE) is self-explanatory; the embryo or fetus is exposed to alcohol (usually ethanol) in utero following maternal ingestion. Fetal alcohol spectrum disorder (FASD) is a constellation of clinical neurological and/or malformative abnormalities that are associated with PNAE. PNAE is easy to define, but not easy to quantify or document with certainty. FASD has been difficult to define to the satisfaction of all. Several methods have been and are used to diagnose FASD using variably weighted criteria related to head and body growth, dysmorphic facial features, neurological development, and documented history of PNAE [1, 2]. At the extremes of stringency, the probability that FASD will be diagnosed in any given individual depends on the tool used [3]. FASD is a subset of PNAE and, because the definitions differ, the pathological features described overlap but are not identical.

The developing central nervous system (CNS) depends on a complex concert of cell proliferation, neuronal/glial fate determination, cell migration, macroscopic configuration change, and axonal growth cone extension [4]. During the embryonic period (up to 8 weeks after fertilization) the major blueprint is formed, and rapid growth with increasing complexity continues during the fetal period. Human brain growth continues after full-term birth, roughly doubling in weight during the first year and eventually tripling in weight (in comparison to newborn weight) by 10–15 years. Postnatal growth is a result mainly of synapse formation and myelination. If the in utero or postnatal environments are suboptimal, postnatal brain development might not occur properly.

General principles of toxicology dictate that the developing brain will be vulnerable to a range of toxins, including ethanol, in a dose-dependent manner [5]. In experimental models the dose and timing of alcohol can be specified. In humans, the precise dose is usually unknown, the dose varies across time, and the toxin(s) interact with other maternal-fetal health factors; therefore, the outcome is unpredictable. Numerous biochemical pathways might contribute to PNAE-associated brain damage including direct toxicity of ethanol on immature brain cells (for example by oxidative free radicals), interference with metabolic pathways (e.g. folate), and epigenetic modifications. A consideration of the postulated molecular pathogenesis is addressed in other chapters.

Some aspects of the purported neuropathology of PNAE have been extrapolated from magnetic resonance (MR) imaging studies of individuals diagnosed with FASD. The most common abnormality is reduced brain volume, often with apparent selective vulnerability of certain deep gray

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matter structures [6–8]. Imaging studies of brain morphology can be designed to provide good comparisons between FASD cases and controls. Autopsies, while suffering from ascertainment and selection bias, offer a specificity of pathologic and cytologic detail that in vivo imaging cannot. Information derived from autopsies can also be used to guide the development and interpretation of animal models.

Review of PNAE/FASD Neuropathology

Beginning c2015, we have used a continuously updated broad approach (i.e. not relying on narrow Boolean descriptors) to search PubMed, EMBASE, and GoogleScholar for autopsy descriptions of brains from subjects with PNAE / FASD [9]. The summarized information is shown in Table 26.1. The most commonly reported abnormalities are small brain (microencephaly), deficiencies of the corpus callosum, glioneuronal heterotopia (subpial and periventricular), hydrocephalus, focal cortical dysgenesis, subtle vascular abnormalities, and holoprosencephaly. Unfortunately, most of the reports consist of single cases or small series, which emphasize the most dramatic abnormalities.

Our recently published series of 174 PNAE /FASD cases [9] shows that the brain is small in ~18%. Acquired vascular lesions (ischemic or hemorrhagic) that occur in utero or as a complication of prematurity are the most common focal abnormalities. Abnormalities of maldevelopment including neural tube defects, hydrocephalus due to aqueduct stenosis, corpus callosum defects (3/5 in combination with complex brain anomalies), and leptomeningeal heterotopia occurred in a minority (Table 26.2). Among the cases where information was available, 86% of the mothers smoked tobacco during the pregnancy and 77% abused other illicit drugs. We are

Table 26.1 Reports (in chronological order) of autopsy brain pathology in humans with documented PNAE or clinical diagnosis of FASD

Author(s) / Year	Primary reference	Number of Cases	Age at death	Sex	Major neuropathologic findings	Additional case details and comments
Jones & Smith 1973	[10]	1	32 weeks gestation premature birth +5 days	Female	Microencephaly (140 g); lissencephaly; extensive leptomeningeal heterotopia on dorsal cerebrum (bridging midline); absent corpus callosum; periventricular heterotopia; dysplastic inferior olivary nuclei	Case 2 in the publication. Well-documented maternal alcohol intake. Face, limb, and heart anomalies. Gross photos of this case were not originally shown, but later appeared in several publications [11, 12]. In one publication, the child was reported to have died at 6 weeks of age [13]. A figure from Clarren 1986 comparing this brain to a normal brain [14] appears on many internet sites, where it is alleged to be a typical FAS brain. It is, in fact, an extreme example.
Clarren 1977	[15]	1	Born at term +10 weeks	Male	Brain normal weight (660 g); hydrocephalus (moderate) caused by leptomeningeal heterotopia around brainstem; absent brainstem nuclei; hypoplastic cerebellum and pons	Case 1 in this document is the same case published by Jones [10]. The new case in this publication is also described elsewhere [13]. Hypoplastic pons and cerebellum were reported in a neonate with FAS using MR imaging [16].
Peiffer et al. 1979	[17]	6	17 weeks gestation	Not indicated	Microdysplasia of brainstem nuclei; leptomeningeal lipoma adjacent to medulla oblongata; cardiac anomaly	The same cases are described in less detail in two other publications [18, 19].
Ibid			18 weeks gestation	Not indicated	Microdysplasia of dentate nuclei and inferior olivary nuclei	Well-documented alcohol intake.
Ibid			20 weeks gestation stillbirth	Not indicated	Hydranencephaly	Well-documented alcohol intake plus clomethiazole, a suspected teratogen [20].

Table 26.1 (continued)

Author(s) / Year	Primary reference	Number of Cases	Age at death	Sex	Major neuropathologic findings	Additional case details and comments
Ibid			6 months postnatal	Male	Mild microencephaly (640 g); mild hydrocephalus; "retarded maturation" of white matter; heterotopic cerebellar gyri near the tonsils; lumbar spina bifida occulta	Well-documented drinking during pregnancy. Multiple congenital anomalies including heart defect.
Ibid			9 months	Female	Microencephaly (840 g); polymicrogyria, absent olfactory bulbs; severe hydrocephalus with rupture of occipital lobes; agenesis of corpus callosum; occipital meningocele with associated cerebellar abnormalities; hydromyelia / syringomyelia	Polymicrogyria is not illustrated; note that severe hydrocephalus can be associated with the appearance of polygyria. From the published photographs it appears that the corpus callosum might have been destroyed by the severe ventriculomegaly (i.e. not agenesis).
Ibid			4 ½ years	Male	Normal size brain (1280 g); many ectopic neurons in white matter; partial fusion of thalami	Congenital heart defect.
Clarren et al. 1978	[13]	2	29 weeks gestation stillbirth	Male	Mild hydrocephalus with "rudimentary brainstem and cerebellum"	Cases #1 and #2 in this publication were previously described [10, 15]. Weekly binge drinking in this case. Facial and cardiac anomalies.
Ibid			30 weeks gestation +3 days	Not indicated	Brain weight normal (210 g); 5 mm neuroglial heterotopia on cerebellar folium	Well-documented maternal alcohol intake—multiple binges per week.
Kinney et al. 1980	[21]	1	35 weeks gestation premature birth +3 months	Male	Microencephaly (300 g); partial agenesis of corpus callosum.	Well-documented maternal drinking. Multiple anomalies including diaphragmatic hernia. Paravertebral neuroblastoma (1.5 cm).
Clarren 1981	[22]	1	4 years	Female	"Brain showed small size (weight not reported), marked reduction in cerebral white matter, and neuronal heterotopias along the lateral ventricular surfaces"	Child with clinical diagnosis of FAS died in traumatic accident. Illustration shows hydrocephalus, not "greatly reduced white matter" as claimed.
Majewski 1981	[19]	1	17–20 weeks gestation	Not indicated	Normal	Seven cases are briefly described, the 6 abnormal ones were previously described in greater detail [17].
Van Rensburg 1981	[23]	1	Term birth +1 day	Female	Probable microencephaly (head 28 cm; <third percentile); "brain was histologically normal"	Well-documented heavy maternal drinking. Severe phocomelia of all limbs, facial anomalies.
Wisniewski et al. 1983	[24]	5	8 months	Female	Severe microencephaly (450 g); absent corpus callosum; underdeveloped cerebellar vermis; heterotopic glial clusters in the meninges	Maternal history of heavy drinking is well documented in all cases. Except for the 29-week case, all had cardiac anomalies. This case is also described elsewhere [25].

(continued)

Table 26.1 (continued)

Author(s) / Year	Primary reference	Number of Cases	Age at death	Sex	Major neuropathologic findings	Additional case details and comments
Ibid			32 weeks gestation +4 months	Male	Microencephaly (395 g); shallow sulci with heterotopic glial clusters in leptomeninges causing gyral fusion	In another publication, the brain weight is reported as 359 g [25].
Ibid			Term birth +2 days	Male	Microencephaly (263 g); small meningeal glial heterotopic protrusions forming bridges between adjacent gyri	
Ibid			35 weeks gestation +17 days	Male	"Heavy but small" brain (405 g); single "small glio-neuronal meningeal heterotopia"	Ischemic changes (white matter necrosis) and edema following 3 days survival after cardiac resuscitation.
Ibid			29 weeks gestation +4 days	Male	Small brain (124 g); single heterotopia in temporal lobe white matter	
Ibid		6	Stillbirths	Not indicated	Authors wrote, "six products of miscarriage of drinking mothers which we had examined previously (Byrne et al. in preparation) did not display gross brain malformations."	We could not find evidence that this had ever been published.
Adebahr & Erkrath, 1984	[26]	1	4 months	Male	Slightly small brain (472 g); small supernumerary mammillary bodies lateral to normal structures;	Born prematurely "at end of eighth month"; authors also reported "indentations" of the external granular layer of the cerebellum, but the illustration is not convincing of a true abnormality.
Pratt & Doshi 1984	[27]	1	21 months	Female	Severe microencephaly (684 g); heterotopic neurons and microcalcification in white matter	It is not clear what this case represents; authors wrote, "possible case of FAS", "enquiries failed to elicit a history of maternal drinking".
Ferrer & Galofre 1987	[28]	1	4 months	Male	Microencephaly (440 g); small frontal lobes with exposed insula; irregular gyri; "discrete enlargement of the lateral ventricles"; Golgi staining showed simplified dendrites of cortex layer 5 neurons	Well documented heavy maternal drinking. Facial and cardiac anomalies. It is not clear if "discrete enlargement of the lateral ventricles" means mild. The published image shows a distinct vertical groove extending upward from the Sylvian fissure; it is not clear if this is an abnormal sulcus of a schizencephalic cleft.
Bonneman & Meinecke 1990	[29]	1	31 weeks gestation stillbirth	Male	Holoprosencephaly with cyclopia and agnathia	Normal male karyotype.
Ronen & Andrews 1991	[30]	1	28 weeks gestation premature birth +8 days	Not indicated	Holoprosencephaly (alobar)	Mother was heavy drinker, smoker, and took chlordiazepoxide and imipramine during pregnancy. Authors also reported 2 other cases of semilobar holoprosencephaly without autopsy.

Table 26.1 (continued)

Author(s) / Year	Primary reference	Number of Cases	Age at death	Sex	Major neuropathologic findings	Additional case details and comments
Coulter et al. 1993	[31]	1	37 weeks gestation +2.5 months	Female	"Incomplete" (i.e. semilobar) holoprosencephaly with microencephaly (293 g); diffuse and patchy loss of Purkinje neurons	Maternal binge drinking during first trimester. Very detailed description.
Norman et al. 1995	[32]	3	Not specified	Not indicated	Microencephaly without other malformations	All had clinical diagnosis of FAS, but no details published. The authors noted, "it is apparent that the large majority of surviving children with FAS do not have gross morphologic abnormalities".
Ibid			Not specified	Not indicated	Normal	"Few small calcifications in centrum semiovale" is nonspecific.
Ibid			Not specified	Not indicated	Complex malformation with: Abnormal lamination of cerebral cortex; heterotopic gray matter at the ventricle surface; Dandy-Walker malformation; calcification of the brainstem	
Konovalov et al. 1997	[33]	47	5–12 weeks gestation embryos and early fetuses (n = 44); 14–15 weeks gestation fetuses (n = 3); terminated pregnancies from alcoholic women (+ 16 cases from non-alcoholic mothers)	Not indicated	Abnormalities in 74% of cases (allegedly dose dependent); 25 cases with abnormal "folding" and invagination of the neuroepithelium which might be precursors of microgyria or heterotopia; 2 cases agenesis of olfactory bulbs; 2 cases agenesis of epiphysis; 1 case agenesis of optic chiasm and visual tracts; 6 cases "dysraphia" at midbrain level; 1 case cerebellum agenesis; 7 cases cerebellum dysplasia	The same cases are described in a series of publications in Russian [34–39]. Inspection of the published micrographs suggests that most of these alleged abnormalities are likely artifactual. Convoluted cortical surfaces [33, 34] might be due to autolysis. The cerebral distortions in many cases [36] appear to be folding artifacts. Many of the examples of midbrain dysraphia [38] appear to be cracks or folds in the tissue. Other reported abnormalities are not illustrated. Therefore, this set of publications likely exaggerates the abnormalities in the human embryo and early fetus.
Wang et al. 2006	[40]	Approximately 20	18–22 weeks gestation fetuses; terminated pregnancies from women with histories of cannabis or alcohol ingestion and controls	Not indicated	No pathology described	Gene expression analysis; PNAE associated with decreased opioid kappa receptor expression.

(continued)

Table 26.1 (continued)

Author(s) / Year	Primary reference	Number of Cases	Age at death	Sex	Major neuropathologic findings	Additional case details and comments
Solonskii et al. 2008	[41]	23	10–12 weeks gestation fetuses; terminated pregnancies from alcoholic women (+ additional controls)	Not indicated	Light and electron microscopic analysis of neocortical blood vessels showed increased density of abnormally small blood vessels at 10–12 weeks compared to controls. Authors speculate that abnormal vasculogenesis might be a response to hypoxia, secondary to placental dysfunction.	Macroscopic abnormalities (if any) were not described. Samples of unspecified brain parts were fixed in glutaraldehyde. Illustrations are sparse, we are not very confident in the validity of these data. The same cases are described in a series of publications in Russian [42, 43]. Ultrastructural features of abnormal mitochondria were claimed; however, there are no illustrations so we cannot determine the veracity [44].
Jégou et al. 2012	[45]	11	20 to 38 weeks gestation	Not indicated	Microscopic analysis of neocortical blood vessels showed disorganization of branching (but no difference in density) in fetuses >30 weeks compared to controls	In Table 26.2, summary information documents no malformations in nine cases; three cases with brain weight at or below fifth percentile (including the 38 week case described below); one 33 week case with "diffuse astrogliosis, decreased cellular density into [sic] ganglionic eminences"; and one 38 week case with "white matter heterotopic neurons, microcephaly, inferior olivary complex pachyria [sic]".
Stoos et al. 2015	[46]	1	34 year old	Female	Brain weight at low end of normal range (1150 g). Focal dysgenesis with enlarged right superior temporal gyrus; focal abnormal neuron columnar organization	Clinical diagnosis of FAS with facial anomalies and chronic cognitive impairment. Died from pulmonary embolus.
Sarnat et al. 2015	[47]	1	Term birth +1 day	Male	Bilateral areas of cortical pachygyria and polymicrogyria; synaptophysin immunoreactivity in frontal cortex comparable to 33 weeks gestation	Microcephaly (head size not specified) and "typical facies". Possible delayed synaptogenesis.
Tangsermkijsakul 2016	[48]	1	6 months	Male	Microencephaly (400 g); agenesis of corpus callosum	Died from aspiration pneumonia. Abnormal facial features, head 36 cm (<tenth percentile).
Oza et al. 2016	[49]	1	27 weeks premature birth +7 weeks	Male	Three separate intracranial hemangiomas	Dysmorphic face, multiple vertebral segmentation abnormalities, coarctation of the aortic and bicuspid aortic valve; death due to bronchopulmonary dysplasia
Jarmasz et al. 2017	[9]	174	20 weeks gestation fetus to 65 years	Both	See Table 26.2.	See publication and the associated Supplement for details.
Wygant 2019	[50]	1	8 years	Male	Bilateral areas of cerebral infarction sustained in utero + moderate ventriculomegaly	"History of fetal alcohol syndrome, In utero cerebral infarcts, and infantile autism"; death from intestinal obstruction.

Table 26.1 (continued)

Author(s) / Year	Primary reference	Number of Cases	Age at death	Sex	Major neuropathologic findings	Additional case details and comments
Del Bigio 2021	This report	1	23 weeks gestation stillbirth	Male	Gross and microscopic features consistent with normal development.	Mother used alcohol and marijuana during the pregnancy, with poor prenatal care. No malformations.
Ibid		1	27 weeks premature	Female	Sacral lipoma	Mother drank throughout pregnancy; amniotic band with chest / abdominal wall defect and externalized heart/abdominal organs.
Ibid		1	28 weeks premature	Male	Leptomeningeal, cortical, and periventricular inflammation; large left intracerebral hemorrhage and bilateral intraventricular hemorrhage	Mother drank heavily throughout pregnancy; abnormalities suggestive of in utero infection, but a specific agent could not be proven.
Ibid		1	35 weeks premature	Female	Microencephaly (brain 129 g; <third percentile) with: Focal ventricle wall fusion, rare periventricular heterotopia, retarded laminar development of cerebral cortex, severe pontine hypoplasia.	Mother has FAS, drank heavily during pregnancy; severe fetal growth restriction (body 843 g; <third percentile), facial abnormalities, complex cardiac defect, and small placenta (<third percentile). An underlying genetic abnormality might contribute to the severe anomalies.

Table 26.2 Summary of brain abnormalities in 174 autopsy cases with PNAE (stillbirths and infants) or clinical diagnosis of FASD

	Stillbirths	Infants	Children	Teenage	Adults	% of all cases
Total examined	52	65	32	14	11	
Age range	20 to 41 weeks gestation	>1 day to 12 months (includes 24 premature)	13 months to 12 years	13 to 19 years	20 to 65 years	
Median age	32 weeks gestation	5 weeks postnatal	3.5 years	15.5 years	25 years	
Microencephaly (<fifth percentile brain weight)	11	8	6	1	6	18.4%
Neural tube defects	4	2	–	–	–	3.4%
Corpus callosum dysgenesis + hydrocephalus	1	2	–	–	–	1.7%
Corpus callosum dysgenesis + other minor anomaly	–	1	1	–	–	1.1%
Hydrocephalus (simple)	–	2	3	–	1	3.4%
Minor leptomeningeal heterotopia	–	6	1	–	–	4.0%
Regional hypoxic-ischemic or hemorrhagic lesions unrelated to prematurity	1	1	4	–	–	3.4%
Vascular brain lesions related to prematurity	–	2	4	1	–	4.0%
Probable genetic brain malformation	1	–	1	–	–	1.1%
Mesial temporal sclerosis	–	–	3	–	–	1.7%

Cases from Health Sciences Centre Winnipeg; 1980–2016.
Modified from Jarmasz et al. 2017 [9]; Open Access article under the terms of the Creative Commons Attribution Non-Commercial License

currently trying to determine in a narrow subpopulation of cases with a clinical diagnosis of FASD and microcephaly if there are abnormalities in the distribution of cortical, striatal, and cerebellar neuron populations; preliminary data suggest that any difference from age-matched controls is minor.

Discussion

Alcohol Dose and Multiple Substance Abuse

Uncertainty about the magnitude (i.e. the dose and timing of alcohol ingestion) of PNAE is ubiquitous in autopsy studies [51]. The Safe-Passage Study of 11,892 pregnancies comes closest to a prospective documentation of alcohol ingestion during pregnancy [52]. Drinking behaviors were divided into five patterns based on drinks per day in each month of pregnancy (high early, quit later; high continuous; moderate early, quit later; low continuous; none) [53]. There was a statistically significant trend between drinking and tobacco smoking; continuous drinkers were more likely to smoke in higher quantities (~85%) [54]. Both alcohol and tobacco exposure in utero were associated with abnormal electroencephalography activity in neonates [55]. Prenatal alcohol and tobacco appear to work synergistically to increase the risk of preterm labor [56] and are associated with a much higher risk for sudden death in infancy. Of the 77 deaths in infancy, 28 were classified as sudden infant death syndrome (SIDS) and 38 had a known cause of death (among these: congenital rubella in 1, and brain malformation or multiple congenital defects in 2) [57]. Detailed studies of neuropathology are not yet available from this cohort.

Thorough epidemiologic studies show that PNAE is seldom a single toxin exposure. Almost all mothers of offspring with high PNAE also smoked tobacco products (which can affect placental blood flow and oxygen delivery) [57], a large proportion also abused other drugs (polysubstance abuse) [58–62], and approximately half of the mothers had inadequate prenatal care, possibly including poor nutrition [63]. Alcohol and tobacco use during pregnancy both have the capacity to restrict fetal growth, likely through separate mechanisms [64].

Note that the vast majority of experimental animal studies are confined to alcohol alone at defined time points. Although these experiments can show the damage PNAE is capable of causing, these alcohol-only experiments may not be a good benchmark for the human neuropathology.

Microencephaly

Autopsy and imaging studies show that PNAE / FASD are associated with malformations of the CNS. Microcephaly is one of the criteria used for diagnosis of FASD [3]. Although reduced brain volume has been demonstrated using MR imaging on PNAE and FASD cases [65, 66], the relationship with head circumference is not strong [67]. In our large study of PNAE, the most common abnormality was microencephaly [9]. Generalized reduction in brain volume in early fetal development is likely related to reduced cell content through diminished proliferation or increased cell death or both [68–71]. Reduced brain volume in infancy and later could be a consequence of reduced cell content, or might be due to decreased neuron maturation (i.e. synaptogenesis) [28, 72, 73]. Although myelin formation also accounts for considerable post-natal brain growth [74], there is no evidence for abnormal myelination in autopsy studies of PNAE/FASD brains and the evidence from MR imaging studies is weak [75].

Neural Tube Defects

Neural tube defects were observed in our study of PNAE autopsies and have been previously reported in FAS cases [76, 77]. This malformation occurs during the fourth week after conception [78]. Experimental animal studies support the possibility that alcohol can interfere with neural tube closure [79, 80]. However, several large epidemiologic studies and a recent meta-analysis do not support an association between PNAE and neural tube defects in humans [81–83]. Nutrition may be the major determinant of these malformations [84].

Vascular Damage

Irrespective of the possible direct effects on CNS development, PNAE can create other problems that might have downstream adverse effects on the brain. PNAE increases the probability of premature delivery or complicated labor [58], which are associated with a range of neurological complications including germinal matrix/intraventricular hemorrhage and hypoxic-ischemic brain damage [85–87]. Among women who are intoxicated at the time of labor and delivery, about 18% were reported to have a stillbirth and 30% of the children died in infancy [88]. Ischemic and hemorrhagic brain lesions have been observed fairly frequently in the PNAE population. Some appear to occur in utero and others occur as a consequence of premature birth. The risk factors for focal arterial ischemic lesions acquired in utero (frequently referred to as schizencephaly in the imaging literature) [85] are young maternal age, lack of prenatal care, and PNAE [89]. PNAE research using large animal models has convincingly shown that cerebral circulation is altered [90, 91]. Alcohol and tobacco combine to interfere with uterine oxygen delivery [92]. Ethanol infusion suppresses cerebral oxidative metabolism to a greater extent than would be

expected based on the decrease in placental blood flow [93, 94]. Baboon experiments show that alcohol causes dilatation of fetal cerebral arteries may at least transiently interfere with autoregulation [95] and that placental perfusion is significantly impaired [96]. Sheep experiments show that cerebral arteries are stiffer after PNAE [97] and that altered cerebrovascular reactivity can persist from the fetal period into adulthood [98–100]. Repeated PNAE late in pregnancy was associated with small intracerebral hemorrhages in fetal sheep [101]. In comparison to the functional vascular disturbances, actual developmental effects on the vasculature are less clear. The density of the microvasculature (i.e. the capillary network) is not substantially altered in fetal sheep with PNAE [102]. However, the radial organization of cortical microvessels was reportedly abnormal in brains of late-gestation human fetuses with PNAE [45].

Among the alcohol-related birth defects, congenital cardiac anomalies such as tetralogy of Fallot are known to occur [103, 104]. These can have secondary effects on the brain. In autopsy studies, up to 60% of infants and children with cyanotic heart malformations have associated acquired brain abnormalities including hemorrhage, hippocampal damage, and diffuse white matter damage [105, 106].

Corpus Callosum Anomalies

Corpus callosum deficiencies of varied extent (partial or complete agenesis) are regularly reported in autopsy and imaging studies of PNAE cases [107, 108]. In humans, the hippocampal commissure appears at 10–11 weeks gestation. Pioneer axons of the anterior part of the corpus callosum appear at 11–13 weeks gestation. Rostral and caudal extension of the corpus callosum occurs as the cerebral hemispheres expand, reaching its full extent by ~17 weeks [4, 109, 110]. Pioneer axons from the cingulate cortex serve as a guide for later arriving neocortical axons, which reach a maximum at ~32 weeks gestation, and contribute to the expansion of the corpus callosum [111, 112]. Among newborns with a diagnosis of FAS (90% of whose mothers also smoked tobacco), MR imaging showed an average smaller cross sectional area of the corpus callosum even after controlling for intracranial volume [113]. Rodent models of PNAE / FASD have shown at most only subtle changes in the volume (but no abnormalities in the extent) of the corpus callosum [114–117]. The apparent discrepancy with human data could be related to the timing of the experimental PNAE. One primate experiment showed an increase in the quantity of callosal axons [118]; this might be due to a failure of retraction of callosal axons, which are produced exuberantly [111].

If alcohol plays a teratogenic role in the corpus callosum, there are at least two periods of vulnerability. From 11–17 weeks, interference with pioneer axons could result in dysgenesis of the corpus callosum. In the latter half of fetal development, growth of the second wave of neocortical axons, might be compromised. There is some evidence in cell culture that ethanol can alter axonal growth cone responses to guidance cues [119], but this line of research is not substantial. If PNAE affects layer 3 neocortical neuron populations, the initial outgrowth of neocortical callosal axons might be reduced.

It is not entirely clear to what extent PNAE is a true risk factor for callosal dysgenesis, because the abnormality can be seen in a wide range of situations [120] and might merely be incidental. Simple notches in the dorsal corpus callosum are not part of FASD [121] and are most likely related to impingement by the falx cerebri.

Hydrocephalus

PNAE has been identified as probable risk factor for congenital hydrocephalus [122]. Hydrocephalus due to aqueduct stenosis was among the most common abnormalities in our autopsy study. However, the primary failure of aqueduct patency has never been demonstrated in any situation, and the timing of this anomaly is likely inconstant. Aqueduct stenosis has varied morphology, from an abnormally small but otherwise patent lumen to an obviously scarred region; the pathogenesis is seldom clear [123, 124]. In 1914 Dandy and Blackfan proposed an infectious etiology for aqueduct stenosis [125], while later authors favored a 'developmental' origin without specifying a mechanism [126, 127]. Mice with mutations that affect the integrity of the ependymal lining [128] or the function of ependymal cilia [129] are predisposed to hydrocephalus. However, hydrocephalus is a rare outcome in PNAE animal models [130] and there is no good evidence for either of these ependyma injury phenomena following PNAE in humans. Patchy ependymal inflammation and damage secondary to subtle in utero infection or hemorrhage is the likely cause of aqueduct stenosis in at least some cases [131–135]. Similarly, there is some evidence that scattered minor periventricular heterotopia (in contrast to multiple large nodules) can occur as a consequence of in utero infection [136, 137]. Despite the likelihood that infectious processes occur, one cannot absolutely exclude an acquired or incidental genetic abnormality; exome sequencing reveals potentially pathogenic mutations in ~20% of sporadic congenital hydrocephalus [138].

In Utero and Neonatal Infections

Women who drink during pregnancy may be at increased risk for syphilis, HIV, rubella, toxoplasma, and hospital-acquired infections [139–142]. PNAE is associated with a

2.5-fold increased risk of subsequent neonatal infection [143, 144], possibly because of adverse effects on the developing immune system [145]. Thus, PNAE may increase the risk of brain damage ranging from classic congenital infections to the results of more subtle infections. As noted in the section above, some subtle infectious processes can cause brain damage that might be attributed to PNAE irrespective of a direct relationship. More controversially, a maternal "inflammatory environment" is postulated to interfere with normal fetal brain development [146, 147].

Shortcomings of Autopsy Studies

Autopsy studies are subject to severe selection and ascertainment biases. Cases of early death or grossly abnormal brain are more likely to be selected for autopsy. The nature of deaths in the context of PNAE or FASD makes the deaths far more likely to fall into the medico-legal realm. It is very difficult to conduct research using medico-legal autopsies because of the jurisdictional mandates. Most studies are retrospective and are limited by the variable and often inadequate brain tissue sampling. Regardless of the sampling, it is difficult to evaluate subtle neuronal changes, which might be associated with significant neurological or behavioral abnormalities. The autonomic nervous system, which is hypothesized to be dysfunctional in FASD [148], is almost never examined in detail. The information about alcohol exposure is typically limited and differs according to the age at death [9]. Following fetal or neonatal deaths, mothers or guardians will typically be asked directly about alcohol, drug, and tobacco exposure. Deaths in children and adults with a clinical diagnosis of FASD will typically lack detailed PNAE information. PNAE cases with severe neurological abnormalities due to destructive brain lesions and prematurity-associated brain damage are much less likely to be considered in the FASD diagnostic categories (personal communication Ana Hanlon-Dearman, Medical Director Manitoba FASD Centre).

Conclusions

Although high dose PNAE increases the risk for brain malformations and brain damage in humans, a precise or accurate cause-effect relationship between PNAE and any brain anomaly cannot be ascertained. It is highly probable that FASD is the consequence of multiple toxicities (alcohol, tobacco, other drugs) and predispositions (placental and vascular effects, prematurity, infections, nutrition) [9, 149, 150], rather than a primary teratogenic effect of ethanol. Consequently, there is no characteristic or distinctive neuropathological feature of PNAE or FASD, a conclusion that has also been reached in imaging studies [151]. These points are critical in the use of PNAE animal models, which must be interpreted cautiously.

Multiple Choice Questions

1. Studies about prenatal exposure to different drugs, including alcohol and tobacco, have showed:
   A. A clear alcohol-exposure only association with increase in preterm labor incidence.
   B. A clear tobacco exposure-only association with increase in preterm labor and sudden infant death syndrome incidence.
   C. No clear association between drugs exposure during the prenatal period and preterm labor and sudden infant death syndrome incidence.
   D. A synergistic effect is seen with alcohol and tobacco use leading to an increase in preterm labor and sudden infant death syndrome incidence.
   E. A cancelling effect is seen with alcohol and tobacco use, leading to a decrease in preterm labor and sudden infant death syndrome incidence.
   Correct: D
2. Which of the following is wrong about microencephaly in the context of FASD:
   A. Microencephaly is one of the criteria for FASD, even though there is no proven relationship between it and head circumference.
   B. Microencephaly is one of the criteria for FASD, having a strong relationship between it and head circumference.
   C. In some studies of PNAE, one of the most common abnormality was microencephaly
   D. Reduction in brain volume in childhood and later could be the result of reduced cell content, or may be due to reduced neuron maturity.
   E. Reduction in brain volume in early fetal development is likely related to reduced cell content through diminished proliferation or increased cell death or both
   Correct: A
3. Which of these morphologic changes has not been directly or indirectly associated with PNAE and/or FASD:
   A. Corpus callosum deficiency in autopsy studies.
   B. Abnormal myelination.
   C. Microencephaly.
   D. Hydrocephaly.
   E. Ischemic and hemorrhagic brain lesions.
   Correct: B
4. Neural tube defects seen in association with PNAE:
   A. Are result of a direct teratogenic effect of ethanol.
   B. Occur only with exposure to methanol.
   C. Are triggered by ethanol teratogenicity in specific human subpopulations.

D. May be related to nutritional deficiencies, perhaps in association with ethanol exposure itself.
E. Are exaggerated by misinterpreted autopsy findings.
Correct: D

5. The lack of characteristic or distinctive morphologic features of PNAE and/or FASD may be attributed to:
A. Complete lack of progress in the understanding of the neuropathology of PNAE and FASD.
B. The absence of morphologic changes in these pathologies.
C. The fact that the occurrence of FASD is related to the amount and duration of PNAE exclusively.
D. The fact that FASD is a probable consequence of multiple toxicities (alcohol, tobacco and others) and diverse predispositions (placental abnormalities, infectious and nutritional disorders, and others).
E. The fact that FASD is a probable consequence of ethanol toxicity and genetic predisposition.
Correct: D

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Genetics and Epigenetics of FASD

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Learning Objectives

- To understand the evidence that genetics can impact the type and severity of ethanol's teratogenic effects.
- To understand the molecular pathways associated with ethanol-induced cell death, craniofacial dysmorphology, and alterations in neuronal process outgrowth.
- To learn about the different types of epigenetic modifications that are caused by developmental alcohol exposure including DNA methylation and histone modifications.
- To gain an understanding of ethanol-induced changes in microRNA expression and how these could be used as biomarkers of developmental alcohol exposure.
- To gain exposure to the evidence showing that there are transgenerational effects of developmental alcohol exposure.
- To evaluate the types of long-term changes that occur uniquely in adults as a result of prenatal exposure.

Highlights

- Genetics has been shown to impact the type and severity of ethanol's teratogenic actions as shown in human populations, particularly twin studies, and in a variety of animal models including different strains and knockout/overexpression model systems.
- Ethanol affects a wide variety of molecular pathways including expression of members of the apoptotic pathway, and a number of molecules important for craniofacial development.
- Developmental ethanol exposure has effects on a wide range of epigenetic marks including changes in DNA methylation and histone modifications.
- Ethanol exposure has been shown to alter expression of various circulating microRNAs and it may be possible to use the pattern of altered changes as a biomarker of developmental alcohol exposure.
- The evaluation of the effects of ethanol exposure on subsequent generations is just beginning, although all evidence supports the idea that this can occur, and for certain phenotypes, it occurs in a sex-specific manner.
- Developmental ethanol exposure can have unexpected effects in adults that are distinct from the issues caused during childhood including altered stress responsivity, altered immune functioning, and increased incidence of several types of cancers.

Genetic Component to FASD

Evaluation in Humans The determination of whether or not there are genetic effects in humans is complicated by the fact that, in addition to genetic differences, there are a whole host of other differences, ranging from dose and timing of exposure to nutritional status, that contribute to whether or not FASD occurs and the severity of the effects. Moreover, determination of the amount of alcohol that was consumed, and when, is complicated by two factors. First, unless a

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diary of consumption is kept, memory is highly fallible making it likely that amounts and times are misreported. Second, because of the social pressure for women to abstain from alcohol while pregnant, women are often reluctant to admit how much she consumed and/or for how much of the pregnancy. Thus, it is difficult to separate genetic and environmental contributions.

One means of circumventing this issue is through the use of twin studies. In twin studies, of course, the prenatal environment is shared and therefore genetic effects can be evaluated. In twin studies, the concordance of the type and severity of effects is examined in monozygotic twins and compared to the concordance in dizygotic twins. Since monozygotic twins have the same genetic make-up and the same environment, it is expected that the concordance will be high. In contrast, the dizygotic twins will have the same environment but will share an average of 50% of their genetic make-up. If genetics is a significant contributor, it is expected that the concordance level will be lower in dizygotic than monozygotic twins.

There have been a number of twin studies in children with FAS and FASD [1–4]. The results are consistent with a genetic effect as the concordance is high in monozygotic twins at close to 100%, while in dizygotic twins the concordance rate is significantly lower. Thus, it is clear that genetics are a factor in determining the severity of the effects. However, there is a caveat to these results in that, even in comparisons of monozygotic twins, there can be more subtle differences in the type of deficits that are observed, suggesting that there can be modulation by other factors. Therefore, animal models are needed to more clearly evaluate genetic effects.

Evaluation in Animal Models It is in animal models that the effects of genetics are most clearly shown. The advantage of animal models is that the environmental factors are controlled since animals are always examined in the same environment. Additionally, the dose and timing of exposure are controlled by the experimenter eliminating the problems inherent in self-reporting. Genetics are examined by comparing the effects in animals of differing genetic backgrounds (See Call-Out Box 27.1). Examples include mice of different strains, knockouts, or transgenics compared to their respective controls.

Differential sensitivity has been shown for a large number of effects including: major organ systems and skeletal malformations [5–11], craniofacial dysmorphology [12, 13], and changes in gene expression in response to developmental alcohol exposure [12]. Additionally, differential sensitivity has also been shown in a range of phenotypes indicative of central nervous system dysfunction including decreased brain weights [14], differences in growth of the corpus callosum [15, 16], differences in ventricular size [8], differential growth delays in the brain [7, 10], and differences in levels of ethanol-induced cell death [7, 17–19]. There have also been genetic differences in a range of behaviors including hyperactivity, motor incoordination, and social behavior [20–23].

Genetic Pathways Implicated in FASD

Cell Death As discussed in this and other chapters, ethanol exposure during development can cause significant levels of cell death, depending upon the dose and timing of the exposure. Cell death has been classified into several different types including apoptosis, necrosis, and autophagy, although there is overlap between these types at the molecular and cellular levels. Both autophagy and apoptosis as general processes have been studied and specific molecules have been linked to each of these processes. In a number of experiments using animal models, many of these molecules have been evaluated for their role in ethanol-induced cell death (Fig. 27.1a and Table 27.1).

Autophagy is defined as lysosomal-mediated cellular degradation. To date, only a limited number of studies have evaluated the relationship of autophagy and autophagy-related molecules with developmental ethanol exposure, although there are examples of expression changes in these proteins after exposure. For example, one study that identified proteins differentially expressed in the brains of rats exposed to prenatal ethanol exposure showed an altered expression of autophagy-related proteins [24]. This is consistent with previous work that suggested that autophagy may, in fact, provide a protective mechanism for the ethanol-exposed cells and that these pathways may have a potential as therapeutics [25].

However, the strongest link between ethanol exposure and cell death is with apoptosis. Apoptosis has been well studied and both the pro-apoptotic (e.g., *Bax* (Bcl-2 associated X, apoptosis regulator), *Bad* (BCL-2 associated agonist of cell death)), and anti-apoptotic (e.g., *Bcl-2* (BCL-2 apoptosis regulator)) molecules involved in this process have been described. Numerous studies have examined expression of many of the apoptosis-related genes following various ethanol exposure paradigms, and in virtually every study, ethanol has been shown to alter their expression (see Fig. 27.2) [26–29]. As expected, ethanol often alters expression of pro-apoptotic and anti-apoptotic genes in opposite directions leading to the hypothesis that ethanol

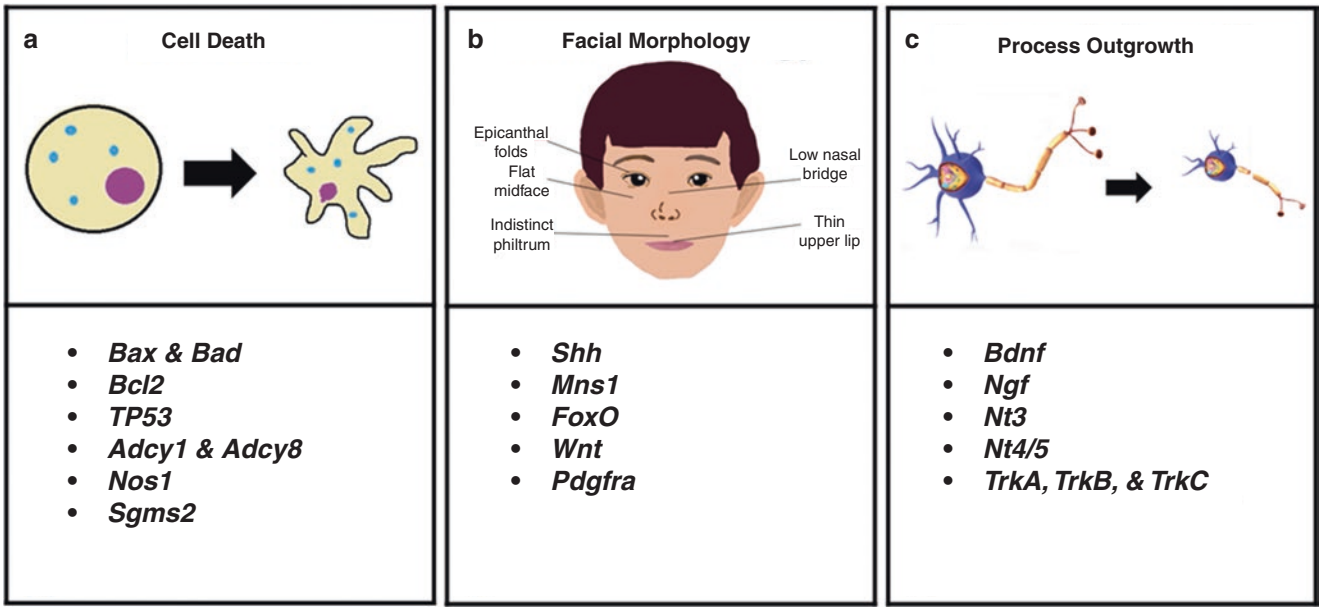

Fig. 27.1 Role of genetics in FASD. Genes that have been shown to be critical in ethanol's effects on cell death, facial dysmorphology, and process outgrowth to show examples where a number of genes and genetic pathways have been identified

Table 27.1 Genes involved in cell death, facial dysmorphology, and process outgrowth after exposure to developmental alcohol

Cell death	
Bax	Bcl-2 associated X, apoptosis regulator
Bad	BCL-2 associated agonist of cell death
Bcl-2	BCL-2 apoptosis regulator
TP53	Tumor protein p53
Nos1	Nitric oxide synthase 1, neuronal
Adcy1	Adenylate cyclase 1
Adcy8	Adenylate cyclase 8
Sgms2	Sphingomyelin synthase 2
Facial dysmorphology	
Shh	Sonic hedgehog
Mns1	Meiosis-specific nuclear structural protein 1
FoxO	Forkhead box O1
Pdgfra	Platelet-derived growth factor receptor alpha
Process outgrowth	
Bdnf	Brain-developed neurotrophic factors
Ngf	Nerve growth factor
NT3	Neurotrophin-3
NT4/5	Neurotrophin-4/5
TrkA	Tropomyosin receptor kinase A
TrkB	Tropomyosin receptor kinase B
TrkC	Tropomyosin receptor kinase C

acts within a cell by dysregulating the balance between these two types of molecules thereby resulting in a cell undergoing apoptosis. Conversely, cells where the balance is not disrupted by the alcohol exposure or where the cells are able to recover from this dysregulation are ultimately able to survive.

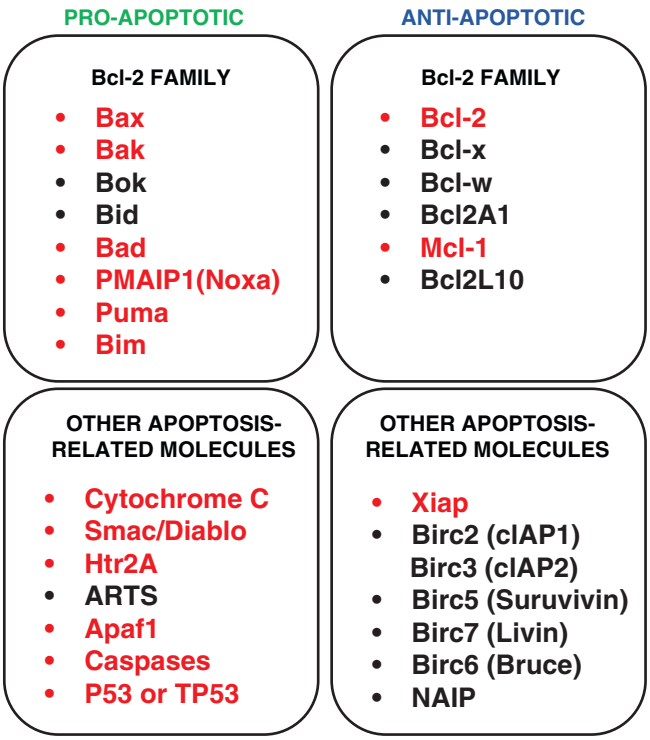

Fig. 27.2 Apoptosis pathways. Examples of pro-apoptotic and anti-apoptotic molecules (genes and/or proteins) that change expression following developmental ethanol exposure. Molecules in red show altered expression, while molecules in black either show no changes or there are no data on expression changes. Whether altered expression is observed, and the direction of the change is dependent on the timing and the dose of ethanol exposure

Evidence has also shown an interaction between genetics and the apoptosis-related pathways, as evaluated using knockout mice or overexpression models. For example, mice deficient in the pro-apoptotic gene *Bax* exhibited less cell death than their wild-type counterparts [30]. Similar results were observed in the examination of another pro-apoptotic gene, *TP53* (tumor protein p53) [31]. Examination of cells that over-express the anti-apoptotic gene, *Bcl-2*, shows that these cells possess enhanced protection from ethanol-induced cell death [32]. These findings suggest that differential expression of apoptotic-related genes could confer sensitivity or resistance to ethanol-induced cell death.

However, the relationship between genetics and apoptosis may be more indirect. Evaluation of other knockout mouse models has also shown that these genes have a role in either enhancing or ameliorating ethanol-induced cell death even though these genes themselves are not part of the apoptosis pathway. Examination of knockouts of the enzyme involved in the synthesis of nitric oxide, *Nos1* (nitric oxide synthase 1, neuronal) [33, 34], adenylate cyclase 1 (*Adcy1*) and 8 (*Adcy8*) [35], or the sphingomyelin synthase 2 (*Sgms2*) [36] have all been shown to alter ethanol-induced apoptosis following developmental ethanol exposure. The link between these genes and the apoptotic pathway remains to be fully elucidated.

Facial Dysmorphology Early gestational alcohol exposure has been linked to distinct craniofacial malformations, including the "classic" facial phenotype of individuals with full-blown FAS characterized by midline defects such as hypotelorism and an absent philtrum, as well as more subtle facial variances. Understanding of the mechanisms by which alcohol disrupts craniofacial development during embryonic stages allows for diagnosis utility and possible future advancement of therapeutic interventions. A number of different pathways have been shown to be important (Fig. 27.1b and Table 27.1).

As shown in mouse embryos, one of the critical molecules in early development of multiple structures is the signaling molecule sonic hedgehog (*Shh*). One consequence of the alterations in the Shh pathway is abnormalities in the formation of the primary cilia [37], and a hallmark of cilia abnormalities are midline facial anomalies (e.g., cleft lips and palates), which are often seen in children with FASD. This pathway may also be important in alcohol-induced disruptions in brain structure. Additional evaluation of molecules important for cilia formation and functioning demonstrates their importance in the facial dysmorphology characteristic of FASD. For example, altered expression of one molecule, *Mns1* (meiosis-specific nuclear structural protein 1, functions in cytoskeletal formation), was found to interact with developmental alcohol exposure to increase susceptibility to ocular defects and related craniofacial anomalies [37].

Other animal models have also been used to identify genes important in ethanol-induced craniofacial dysmorphology. For example, chick embryos have been used to evaluate ethanol-induced changes in gene expression in early-stage chick embryos. The signaling pathways crucial to facial morphogenesis that were shown to be differentially expressed include the transcription factor *FoxO* (forkhead box O1) and members of the *Wnt* signaling pathway [38]. Zebrafish embryos have also been used, and a number of genes involved in ribosome biogenesis were found to be candidates for mediating alcohol's craniofacial teratogenicity. Among the ribosomal genes that are differentially expressed in response to alcohol, lowered expression of ribosomal proteins zrpl11, zrpl5a, and zrps3a was observed [39]. Such a conclusion is consistent with earlier comparisons in chick embryos, where these same candidate genes were found [40].

One further exciting molecule is platelet-derived growth factor receptor alpha (*Pdgfra*). *Pdgfra* was shown to enhance cranio-facial deficits in zebrafish following ethanol exposure. Analyses of *Pdgfra* were subsequently examined in human populations and the link between sensitivity to ethanol-induced craniofacial abnormalities and this gene was strengthened [41].

Process Outgrowths Depending on the time and dose of ethanol exposure, ethanol has been shown to affect the outgrowth of dendrites with both increases and decreases observed. A major pathway involved in dendritic outgrowth is the neurotrophin family and their respective receptors. Neurotrophins belong to a class of neurotrophic factors and are a family of endogenous soluble proteins. Disruption of neurotrophin expression might be involved in the long-term change of memory formation, cognition, and mood alterations that are observed in adults exposed to ethanol during development (Fig. 27.1c and Table 27.1).

The neurotrophin family includes brain-developed neurotrophic factors (*Bdnf*), nerve growth factor (*Ngf*), neurotrophin-3 (*NT3*), neurotrophin-4/5 (*NT4/5*), and their respective tropomyosin receptor kinase receptors (*TrkA*, *TrkB*, and *TrkC*), and these have repeatedly been examined in relation to alcohol-induced alterations in process outgrowth. Studies of prenatal [42, 43] and postnatal [44] alcohol exposure have arrived at the consensus that ethanol exposure decreases BDNF production. Additionally, decreased levels of its receptor, *TrkB*, were also found in both prenatal [43] and postnatal models [45], although there is evidence for sex-specific effects [46]. Furthermore, age-related fluctuations in *Bdnf* gene expression were found in

postnatal exposed models supporting an interaction between alcohol exposure and age on alterations in neurotrophin signaling [47].

Epigenetic Modifications that Occur as a Result of Alcohol Exposure in Humans and Animals

Epigenetics, Development, and Alcohol As stated above, there is evidence that genetic factors can influence the severity of symptoms after developmental alcohol exposure. However, the teratogenic effects of developmental alcohol exposure have also been linked to epigenetic alterations which are gene expression changes that are not due to changes in the genome itself. Rather, epigenetic mechanisms refer to direct modifications of DNA and regulatory factors through DNA and histone modifications and post-translational non-coding (nc)RNAs. Epigenetic processes play a vital role during development and have been associated with cell fate specification and differentiation. During development, epigenetic factors respond not only to a number of internal signaling pathways but can also be altered by exogenous stimuli. These epigenetic modifications during development can be both short-term and long-term, though exposure to negative exogenous stimuli can have detrimental effects on the health of the individual such as described in the fetal programming hypothesis (see Call-Out-Box 27.2). Exposure to alcohol during key developmental events can disrupt normal development and produce fundamental, long-lasting changes in the transcriptome. Epigenetic changes such as DNA methylation, histone modifications, and non-coding (nc)RNA could help explain the variability in FASD phenotypes at the molecular and cellular level in regard to both genetic and environmental factors (Fig. 27.3).

DNA Methylation To date, the most studied epigenetic process is DNA modification, specifically DNA methylation, in which a methyl group (CH3) is covalently attached to the 5′ position of a cysteine, normally at cytosine-guanine dinucleotide (CpG) site. This is accomplished by DNA methyltransferase (DNMT) enzymes that convert cytosine to 5'methylcytosine (5 cM) by adding a methyl group from methyl donors in the folate pathway, specifically S-adenosylmethionine (SAM) (as reviewed in [48]). DNA hypermethylation typically results in repressed gene expression, while DNA hypomethylation is typically associated with enhanced gene expression. In addition, DNA methylation modulates gene expression through direct control of transcription factor binding. DNA methylation is involved in many developmental processes, such as cell fate specification and differentiation, and can be influenced by environmental factors such as alcohol. For example, ethanol interferes with the folate pathway, reducing the availability of SAM to be a methyl donor for DNA methylation [49]. Thus, examination of DNA methylation after exposure to alcohol during development is an important avenue for FASD research.

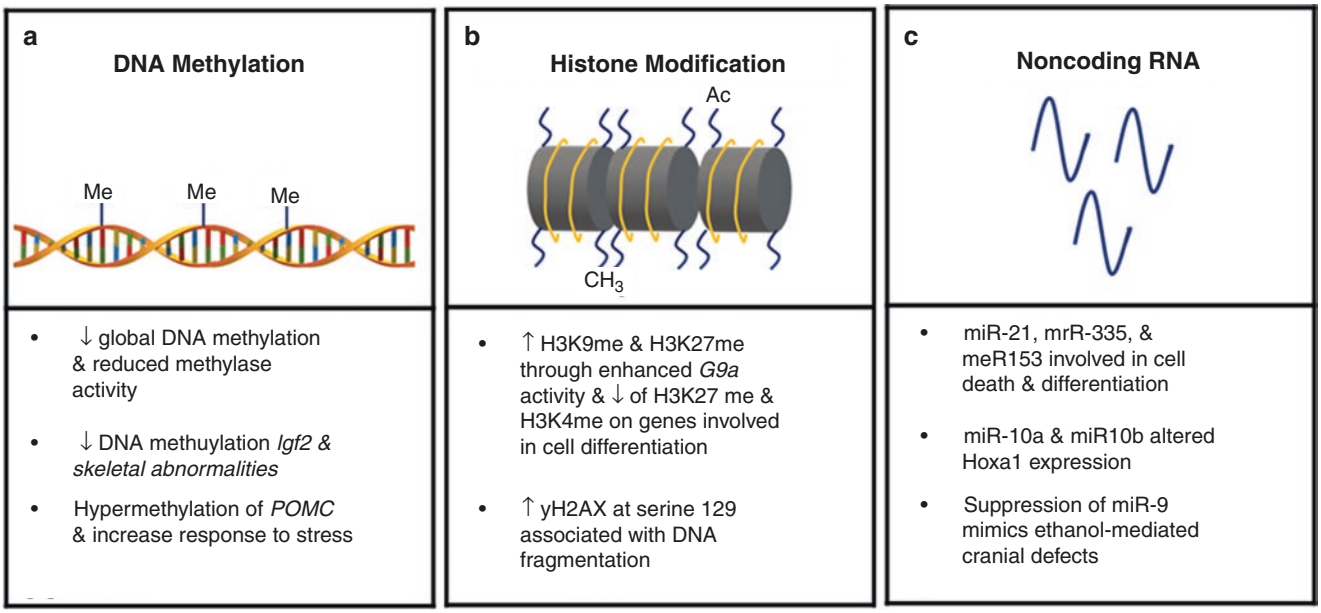

Fig. 27.3 Role of epigenetics in FASD. Examples of epigenetic changes that are observed following developmental ethanol exposure. Changes are observed in DNA methylation, histone modifications, and microRNA expression, which are the major types of epigenetic modifications

The first link between developmental alcohol exposure and DNA methylation was found using a rodent model which discovered decreased global DNA methylation and reduced methylase activity in the hippocampus and prefrontal cortex after exposure to ethanol during mid-gestation (embryonic day 9–11) [50]. Since then, many studies have investigated ethanol-induced alterations on bulk DNA methylation during development and found both increases and decreases in DNA methylation and methylase pathways (Table 27.2) [51–57], suggesting differential alterations in the methylome depending on manner of exposure and region examined (for review see [58]). Other targeted approaches have analyzed methylation levels of specific genes and regions that are methylation-sensitive or implicated in deficits associated with FASD. For example, embryos exposed to alcohol showed decreased DNA methylation and increased expression of the imprinted gene, *Igf2* (insulin-like growth factor 2), as well as skeletal abnormalities [59]. Another group examined long-term methylation and behavioral changes in adult mice developmentally exposed to alcohol and found hypermethylation and reduced expression of *POMC* (proopiomelanocortin) together with increased response to stress [60]. Newer studies are now using genome-wide tools to analyze changes in methylome patterns and coinciding gene expression after developmental alcohol exposure [61]. Genome-wide DNA methylation patterns are also being assessed in children with FASD to provide new avenues for potential targets and possible biomarkers (see Fig. 27.3a and Table 27.2, [56, 57, 62]).

Histone Modifications Another important epigenetic mechanism that has been associated with developmental alcohol exposure is alterations in chromatin structure, particularly those due to histone modifications. Gene expression is influenced by post-translational modifications of histone proteins (e.g., H2, H3, etc.), such as acetylation, methylation, and phosphorylation which determine the genome's accessibility to transcription factors. Generally, histone modifications can be characterized as either associated with increased transcription, such as histone acetylation and histone H3 lysine 4 (H3K4) methylation, or associated with decreased transcription, such as histone H3 lysine 9 (H3K9) and H3 lysine 27 (H3K27) methylation. The effects of developmental alcohol exposure on histone modifications have been understudied; however, emerging research suggests that histone modifications are another important epigenetic mechanism involved in FASD.

The initial evidence of developmental alcohol-induced chromatin alterations found lower levels of the histone core protein H2A in fetal sheep compared to controls [63]. Other genome-wide studies have identified expression differences in many chromatin-associated proteins and histone-modifying enzymes after prenatal exposure to ethanol [64, 65]. In a third-trimester equivalent rodent model, developmental alcohol exposure decreased the bulk level of acetylated histone H3 and H4 in the cerebellum [66]. A separate study, also using a third trimester equivalent rodent model, found increased methylation of H3K9 and H3K27 through enhanced activity of *G9a* (lysine dimethyltransferase) leading to increased cell death in the hippocampus and neocortex after alcohol exposure [67]. Decreases in methylation of H3K27 and H3K4 at promoters of genes involved in cell fate specification and differentiation, as well as decreases in the corresponding gene expression, were found after ethanol exposure in neural stem cells [68]. However, levels of these modifications as well as levels of H3K9 acetylation and H3K9 methylation were dose-dependent with higher levels of ethanol associated with more condensed chromatin and decreased gene expression [68]. Alcohol-induced histone modifications have also been associated with DNA damage.

Table 27.2 Differential alterations to in DNA methylation and methylase activity after developmental alcohol exposure

Type: Bulk						
Direction	Target	Exposure age	Age examined	Tissue	Species	Reference
Decrease	DNA methylation	E9–11	E11	Whole fetus	Mouse	[50]
Decrease	DNA DNMT1 activity	E9–11	E11	HP & PFC	Mouse	[50]
Increase	DNA methylation	P2–10	P21	HP	Rat	[51]
Increase	DNMT activity	E1–22	P21	HP	Rat	[52]
Decrease	DNA methylation	P7	P7	HP & NC	Mouse	[53]
Decrease	Dnmt1, Dnmt3a levels	P7	P7	HP & NC	Mouse	[53]
Increase	DNA methylation	E14.5	N/A	NSC	Mouse	[54]
Type: Genome-wide						
Direction	**Target**	**Exposure age**	**Age examined**	**Tissue**	**Species**	**Reference**
Increase	1028 genes DNA methylation	E8.5	E10	Embryo	Mouse	[55]
Decrease	1136 genes DNA methylation	E8.5	N/A	Embryo	Mouse	[55]
Change	269 differentially methylated CpGs	Confirmed FASD	3–6 years	BEC	Human	[56]
Change	356 hypomethylated CpGs and 302 hypomethylated CpGs	Confirmed PAE	5–18 years	BEC	Human	[57]

Abbreviations: Embryonic day (E), Postnatal day (P), Hippocampus (HP), Prefrontal cortex (PFC), Neocortex (NC), Neural stem cells (NSC), Buccal epithelial cells (BEC)

For example, increased phosphorylation of serine 129 on H2AX was found in the mouse cortex 7 h after developmental alcohol exposure [18]. This histone modification is associated with double-stranded breaks and DNA fragmentation and could act as a marker for apoptosis.

Overall, developmental alcohol exposure is associated with histone modifications that result in condensed chromatin and thus repressed transcriptional activity (further reviewed in [69]). Additional studies are needed to determine the long-term consequences of alcohol-induced histone modifications during development.

Paternal Contribution Because developmental alcohol exposure occurs via the mother, most of the work has focused on the role of the genotype of the embryo and the mother. However, effects of ethanol exposure from the father have been known for over 30 years [70]. Further experiments have examined a range of phenotypes in offspring of ethanol-exposed sires in animal models. Alterations have been found in malformation rates [71], growth rates and metabolism [72], and behaviors, specifically hyperactivity [73]. These changes have been proposed to be due to altered DNA methylation [74] suggesting that changes in the paternal epigenome could play a role in modulating ethanol's teratogenic actions.

Non-Coding Regulatory ncRNAs MicroRNAs (miRNAs) are a group of short, non-coding RNAs. In humans, more than 2000 different miRNAs are expressed, and they have been hypothesized to regulate over a quarter of the expressed mRNAs. miRNAs function by binding to specific sequences in target mRNAs. Binding of the miRNA to the RNA typically inhibits translation of the RNA species into protein usually by initiating a cascade to either sequester or degrade the target mRNA. A specific miRNA typically recognizes a common sequence found in several genes, and thus, a single miRNA can regulate a whole set of different mRNAs making miRNAs especially useful regulators of gene expression. miRNAs have been shown to be regulators of a range of developmental processes associated with cell proliferation, differentiation, and programmed cell death. Further, the presence of circulating microRNAs has been detected in various easily obtained biofluids including serum/plasma, urine, and saliva. This has led to the suggestion that microRNAs may be useful as biomarkers of exposure to various substances and disease states. An assessment of the alcohol-sensitive miRNAs and their regulation and expression provides insight into the mechanisms underlying fetal vulnerability in the maternal–fetal environment following exposure to alcohol intake.

Studies of alcohol-mediated alterations of fetal miRNA expression have mainly focused on changes within the fetal brain. The very first evidence obtained showed that among the miRNAs expressed in the neural stem cells, some were significantly decreased by ethanol exposure [75] including miR-21, miR-335, miR-9, and miR-153. Altered expression of these miRNAs resulted in a decrease in cell death of the neural stem cells and an increase in differentiation of these cells. It has been proposed that the end result of these changes is that ethanol may ultimately drain the pool of neural progenitor cells and promote premature neuronal differentiation thereby resulting in the neuronal loss often observed in individuals with FASD. Similar alterations in miR-9 was were found in zebrafish. In zebrafish embryos, miR-9 expression was found to be down-regulated by ethanol exposure [76]. Suppression of miR-9 mimics ethanol-mediated cranial defects, including microcephaly.

Other miRNAs that have been linked to ethanol-induced alterations are miR10a and 10b. Alcohol exposure during gestation in mice altered expression of miR-10a and miR-10b [77], which in turn, altered expression of *Hoxa1* (homeobox A1). Altered *Hox1a* expression is associated with a number of central nervous system abnormalities suggesting a potential pathway involved in ethanol's neuroteratogenic actions. In another study where zebrafish were employed as the animal model, miRNA expression profiling studies were performed, and a total of 35 miRNA transcripts were significantly differently expressed compared to controls. Among those misexpressed miRNAs, miR-9/9, miR-153c, and miR-204 have CNS expression conserved across humans, zebrafish, and mice. In zebrafish embryos, miR-9 expression was found to be down-regulated by ethanol exposure [76]. Suppression of miR-9 mimics ethanol-mediated cranial defects, including microcephaly.

Several reports have been published in recent years that are examining miRNAs in maternal circulation to assess if there are specific miRNAs that have altered expression following ethanol exposure and therefore could be used as biomarkers. Data from a study across pregnancy show that 11 miRNAs are significantly elevated in plasma from alcohol-exposed mothers and may help to identify individuals who have been exposed to ethanol in utero [78]. Comparisons between changes in serum and plasma show that while there is some overlap, there are also molecules specific to the source. The miRNAs that showed the best ability to discriminate ethanol-exposed and controls were miRs-122, −126, −216b, −221,-3119, −3942-5p, −4704-3p, −4743, −514-5p, and − 602 [79]. Pathway analysis of putative miRNA targets suggested that miRNAs identified in this study are involved in biological pathways that mediate the effects of alcohol, such as brain-derived neurotrophic factor, ERK1/2, and PI3K/AKT signaling.

However, the results of both studies also leave open the possibility that the miRNAs that are significantly altered by alcohol exposure in pregnant woman may not be the same as

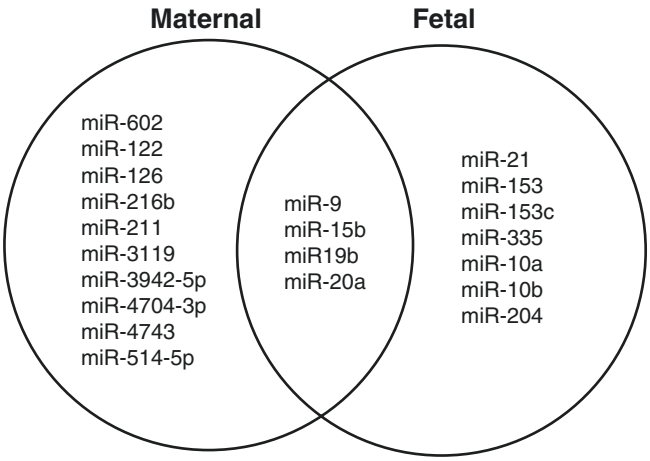

Fig. 27.4 Maternal and fetal miRNA. Venn diagram showing the microRNAs that are differentially expressed in the maternal circulation, in the fetal circulation and showing that both distinct and overlapping microRNAs are found

those which predict infant outcomes (Fig. 27.4). In a binge model of alcohol consumption on pregnant sheep, circulating miRNAs were evaluated in both pregnant ewes and newborn lambs. The profiles of alcohol-exposed ewes and lambs were similar to each other and different from non-pregnant female in plasma, erythrocyte, or leukocyte miRNAs [80]. Circulating miRNAs including miR-9, −15b, −19b, and -20a were sensitive to and specific measures of EtOH exposure in both pregnant ewe and newborn lambs. Shared profiles between pregnant dam and neonate suggest possible maternal–fetal miRNA transfer. This ovine model suggests that circulating miRNAs work as biomarkers for alcohol exposure in both the pregnant mother and the infant.

Transgenerational Effects In addition to the long-term consequences of epigenetic changes caused by developmental exposure to ethanol, epigenetic alterations suggest that there could also be transgenerational changes caused by developmental ethanol exposure. Transgenerational changes are defined as those observed in the F2 or F3 generation (grandchildren/ great-grandchildren) because any changes in the child could be a direct effect of the alcohol exposure and not transmitted. To date, this has not been investigated in human populations, and therefore, it is animal models that have provided evidence examining this issue.

Several animal models have been examined and transgenerational changes have been observed on a number of phenotypes. Exposure of rats to ethanol throughout gestation resulted in diminished learning and memory capabilities as measured on performance in a maze with concomitant alterations in levels of the neurotransmitter acetylcholine (that is closely linked to memory) which was still observed in the F3 generation [81]. Additional hippocampal-associated behavioral deficits were observed from a separate group confirming that transgenerational effects can be observed on multiple behavioral paradigms [82]. A separate study in mice demonstrated that prenatal ethanol exposure altered cortical connections in the offspring given ethanol and that these altered connections were transmitted to the F3 generation and were associated with altered methylation patterns [83].

Several of these differences were observed in a sex-specific manner and, interestingly, were transmitted through the male germline. Several papers have shown in rats that developmental ethanol exposure alters expression of proopiomelanocortin (*POMC*) in the hypothalamus. *POMC* is related to stress reactivity and, as expected, altered stress reactivity is observed in animals exposed to ethanol in utero. The changes are mediated, at least in part, through epigenetic changes and are transmitted into the F3 generation but only in the male germline and not through the female germline [84, 85].

Long-Term Effects of Prenatal Alcohol Exposure

Prenatal alcohol exposure can lead to issues in adulthood such as CNS dysfunction (alterations to the HPA axis and neuroimmune response), immune system dysfunction (arthritis and increased frequency of infections), and breast and pituitary cancers (Fig. 27.5; [86–89]). Each of these maladies has genetic components. Additionally, ethanol exposure can cause epigenetic changes which can, in turn, alter expression of genes associated with each of these conditions and diseases. Data from studies of people with FASD are limited and thus studies using animal models have been used to connect prenatal alcohol exposure with its long-term consequences. However, the study of the long-term effects of FASD is in its infancy, so the data collected thus far is a starting point for future work that suggests a possible link with epigenetic changes.

Alterations to the HPA Axis It has been shown that an increased incidence of anxiety is common among adults with FASD [90]. Connected to this, anxiety has been demonstrated to at least partially result from chronic activation of the stress response [91]. Furthermore, it is well known that anxiety and inadequate stress responses can arise from dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis [87]. The HPA axis regulates the stress response, and since dysfunction of the HPA axis has been shown in animals and human infants exposed to ethanol in utero, the HPA axis in people with FASD potentially exhibits enhanced responsiveness to stress later in life [92]. Furthermore, studies in rodents demonstrate that the effects of prenatal alcohol exposure on the HPA axis can be mediated, at least in part, by the effects

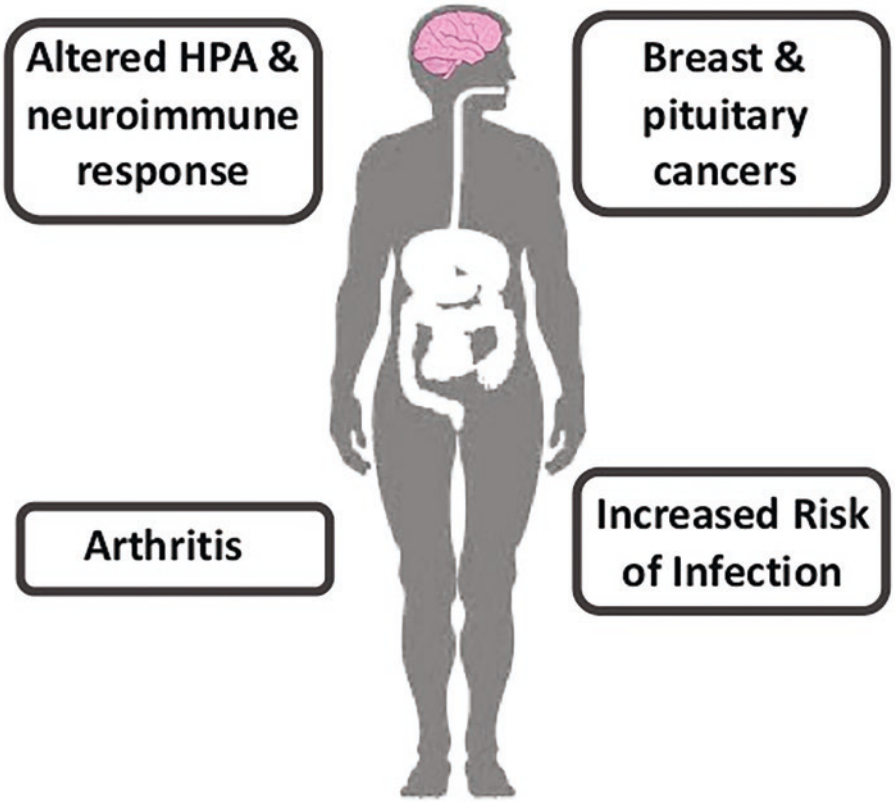

Fig. 27.5 Long-term effects of FASD. Long-term health consequences of developmental ethanol exposure. Individuals with FASD are at an increased risk for a number of maladies as adults. The types of alterations that are currently known are shown in the figure, although it is possible that other types will be identified in the future

of alcohol on DNA methylation [93]. Specifically, several studies have shown that prenatal ethanol exposure in rats results in hypermethylation of the proopiomelanocortin (*Pomc*) gene, providing a potential mechanism of ethanol-induced HPA axis dysfunction [85, 94, 95]. From a functional perspective, *Pomc* codes for a precursor polypeptide for several proteins including melanocyte-stimulating hormones, ACTH, lipotropins, and β-endorphins [94]. Increased methylation of *Pomc*, with the concomitant decreased expression of the gene, can result in decreased amounts of these derivative proteins [96]. Future research on *Pomc* in people with FASD is necessary to further elucidate the potential link between its DNA methylation and psychiatric conditions.

Altered Neuroimmune Response While studies in adults with FASD regarding neuroimmune responses are lacking, animal studies have suggested that there may be lifelong alterations of the neuroimmune system due to prenatal alcohol exposure. Broadly speaking, it has been demonstrated that prenatal alcohol exposure can contribute to microglial activation and loss as well as heightened expression of proinflammatory chemokines and cytokines in a variety of areas of the brain [88]. More specifically, this activation of the microglia by ethanol has been proposed to result in a transition to the proinflammatory phenotype and thus contributes to the loss of neurons [97]. Furthermore, the microglial loss in the brain is linked to a variety of effects such as decreased neurogenesis, neuronal plasticity, and number of synapses [97]. Notably, the activation of microglia and the resulting brain inflammation can result in the same negative impacts as microglial loss [97]. Taken together, alterations in neuroimmune function due to developmental alcohol exposure have the potential to increase the likelihood for subsequent injury due to additional insults [88].

Arthritis It is known that ethanol exposure in utero can result in disruptions of the developing immune system, and one example of a resulting dysfunction is the increased potential for developing forms of arthritis [98]. Since people with FASD are at increased risk for immune system dysfunction, it follows that they may be predisposed to arthritis. In the course of working to understand a potential connection between arthritis and people with FASD, potential mechanisms have been investigated. One possible source is the ethanol-induced alterations of the gene *HLA-DPA1* (major histocompatibility complex, class II, DP beta 1). This gene, which is expressed in the major histocompatibility complex, has been shown to be atypically methylated in people with FASD and thus possibly potentiate rheumatoid arthritis [61, 99]. Furthermore, an animal study on adjuvant-induced arthritis found that prenatal alcohol exposure increased the

length and severity of the condition, which is suggestive of decreased functionality of the immune system [100]. This is a glimpse of the broader idea that prenatal alcohol exposure could potentially contribute to the development of inflammatory conditions and autoimmune diseases due to atypical immune responses [88].

Increased Risk for Infections There is evidence that babies exposed to ethanol in utero have increased frequency of infection and decreased leukocyte counts, which suggests altered immune function throughout life [86]. As a result, animal studies have been done in order to investigate possible root causes such as leukocyte responsiveness and the HPA axis. In a study focused on the immune system of adult mice that had been exposed to alcohol in utero, there was heightened risk and severity of influenza infection due to diminished adaptive immunity and abnormal B-cell responses [86]. Another study focused on the alterations to the HPA axis caused by prenatal alcohol exposure and found that these changes result in greater vulnerability to infectious illnesses [92].

Cancers Studies in humans have not yet investigated the incidence of cancer in adults with FASD. However, there have been animal studies to investigate a potential increase in cancer in adulthood, as well as possible causes, in association with prenatal alcohol exposure. One study suggested an increased risk for breast cancer and pointed to decreased β-endorphin levels and increased stress responses as potential reasons for the inhibition of the immune response against cancer [89]. Furthermore, the incidence of mammary tumorigenesis has been shown to increase in female rats that were exposed to alcohol in utero, potentially via chronic changes in gene expression in the mammary gland due to ethanol [101]. Additionally, an increased risk for pituitary tumorigenesis has been seen in rats with prenatal alcohol exposure, and the hypermethylation and thus decreased expression of the D2R gene in the pituitary has been indicated as a possible source [102].

Call-Out Box 27.1 Study of Genetics Using Animal Models

Animal models have provided a wealth of information on the role of genetics in FASD and in understanding possible molecular mechanism(s) that underlie any genetic differences in phenotypic readouts. Animal models use a variety of techniques each with advantages and disadvantages. Both forward and reverse genetic approaches are used. In forward genetics, a phenotypic difference is observed and then the gene is identified (phenotype to genotype), while in reverse genetics a gene of interest is posited and then analyzed to evaluate its role in a particular phenotype (genotype to phenotype). There are a number of techniques that are used in each approach, although there is considerable crosstalk between approaches. For example, if a gene of interest is identified in a reverse genetic screen, often the same methodologies (e.g., knockout mice) used in a forward genetic scheme will be used to confirm that the proposed gene has the expected role in generating a particular phenotype.

Forward genetics approaches in animal models are analogous to human GWAS studies (genome-wide association studies) in which many individuals are phenotypically examined, and then genotypic analyses were conducted. In this analysis, the phenotype and genotype are correlated to determine the region of interest, and ultimately the gene and DNA variant, that underlies the differential response. There are a number of advantages to the use of this type of approach in animal models. First, the environmental variables can be controlled so that true genetic variation can be elucidated, and false positives minimized. As an example, in human FASD populations, the level of ethanol consumption as well as the timing of exposure varies widely. Therefore, having sufficient sample size in an equivalent population can be challenging to obtain making analyses difficult. Second, a range of animal models is available across numerous species including mice, rats, zebrafish, and drosophila allowing for concurrent analyses across multiple organisms. Third, in animal models, there is control over breeding schemes allowing for rapid evaluation of the role of specific genes. The major disadvantage of this type of approach is that while chromosomal regions can be identified relatively easily, determining the specific gene variant has often been an arduous task. However, recent advances, such as in bioinformatic methodologies, have improved the ability to identify the gene, and these types of experiments in the study of FASD have yielded exciting candidate chromosomal regions and potential candidate genes.

Reverse genetics makes use of a variety of techniques to either eliminate or alter expression of a particular gene or to over-express a specific gene. This technology obviously can only be used in cell lines in human tissue limiting its applicability in the study of FASD, which is truly a multi-organ system disorder in humans. However, animal models have provided a number of candidate genes which are currently being examined in human populations to assess whether these genes confer susceptibility or resistance to etha-

nol's teratogenic effects. Additionally, a number of new technologies may, in the future, improve its applicability. For example, the CRISPR-Cas9 genome editing system has recently been used in tissues from a wide variety of species. In this system, a specific RNA is made to target a particular piece of DNA which is then cut out by the Cas9 enzyme thereby eliminating expression of that gene. This system is efficient and usable in a range of tissues at a range of ages, and therefore, will have great potential in the future. These techniques have been widely used and will ultimately enhance our understanding of the role of specific genes in FASD.

Call-out Box 27.2 Fetal Programming Hypothesis

Part of the Developmental Origins of Health and Disease approach, the fetal programming hypothesis was first theorized in 1993 by Barker who proposed that undernutrition during gestation was an important contributor to adult cardiac and metabolism disorders [103]. This hypothesis suggests that environmental factors during development, such as maternal stress or exposure to toxins, can induce permanent adaptations or perturbations leading to negative cognitive and behavioral phenotypes as well as vulnerability to disease later in life [104]. The mechanism behind the fetal programming hypothesis is not entirely understood, though epigenetic mechanisms are proposed to be involved.

Multiple Choice Questions

1. Question 1: Which of the following evidence from human twin studies supports the idea that genetics plays a role in the severity of the effects observed in FASD?
   a. There is no concordance between the two twins.
   b. Dizygotic twins have a higher concordance than monozygotic twins.
   c. Monozygotic twins have a higher concordance than dizygotic twins.
   d. There is equal concordance between the two twins in both monozygotic and dizygotic twins.
   Correct Answer: C
2. Question 2: Ethanol causes increased cell death:
   a. By enhancing autophagy and not other types of cell death.
   b. By altering expression of the anti-apoptotic gene Bcl-2 and not other genes.
   c. By dysregulating the balance of anti- and pro-apoptotic genes.
   d. By activating the necrosis pathway.
   Correct Answer: C
3. Question 3: MicroRNAs:
   a. Show little altered expression following developmental alcohol exposure.
   b. May be usable as biomarkers of developmental alcohol exposure.
   c. Are only found within the cell.
   d. Increase expression of target mRNAs.
   Correct Answer: B
4. Question 4: Which of the following is NOT an example of an epigenetic change caused by developmental alcohol exposure?
   a. Altered expression of transcription factors
   b. Changes in expression of the core histone protein H2A
   c. Alterations in global DNA methylation levels
   d. Changes in DNA methylation at specific sites in relevant genes
   Correct Answer: A
5. Question 5: Which of the following long-term alterations have been found in adults developmentally exposed to alcohol?
   a. Increased risk of certain cancers
   b. Increased incidence of arthritis
   c. Altered stress responses
   d. All of the above are correct
   Correct Answer: D

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Genetic, Epigenetic, and Environmental Influences on Fetal Alcohol Spectrum Disorder: Implications for Diagnosis, Research and Clinical Practice

Alexandre A. Lussier, Berardino Petrelli, Geoffrey G. Hicks, and Joanne Weinberg

Learning Objectives

By the end of this chapter, readers should be able to:

1. State the prevalence rates of FASD and describe current diagnostic guidelines.
2. Understand evidence-based risk and protective factors that affect FASD outcomes.
3. Identify genes and cell signaling pathways involved in FASD.
4. Discuss epigenetic mechanisms that bridge environmental stimuli and neurodevelopmental outcomes.
5. Connect genetic variation and epigenetic modifications with programmed transcriptomic and proteomic alterations underpinning alcohol-induced deficits.
6. Describe how the identification of new clinically relevant biomarkers of FASD will lead to more accurate and earlier identification of children at risk for FASD.

Highlights

1. Fetal alcohol spectrum disorder (FASD) is a prevalent neurodevelopmental disorder that manifests through a range of cognitive, adaptive, physiological, and neurobiological deficits resulting from prenatal alcohol exposure.
2. The prevalence of FASD is estimated approximately 1–5%, higher than any other neurodevelopmental disorder.
3. The outcomes for individuals with FASD may be influenced by a wide range of environmental factors, including socioeconomic, nutritional, stress, and paternal effects, which can modulate its manifestation.
4. Multiple genetic pathways may mediate the effects of alcohol on development and may increase susceptibility to alcohol-induced deficits.
5. Epigenetic mechanisms may provide a link between the environment and genetic susceptibility to alcohol, while acting as potential biomarkers of FASD.
6. Transcriptomic and proteomic alterations can influence the outcomes in individuals prenatally exposed to alcohol and may act as potential biomarkers of FASD.

Introduction

The adverse effects of prenatal alcohol exposure on offspring development were first described in papers by Lemoine and colleagues [1] and then by Jones and colleagues [2], who

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coined the term Fetal Alcohol Syndrome (FAS). The key observations by both groups included pre- and post-natal growth deficiencies, minor facial abnormalities, and damage to the developing brain that could result in behavioral, cognitive, attention, and adaptive alterations. Since then, thousands of clinical studies have confirmed and considerably extended these initial findings, describing deficits across multiple physical, physiological and neurobiological domains [3–5]. The term fetal alcohol spectrum disorder (FASD) was first introduced around the year 2000 to recognize more specifically this broad spectrum of effects, and was formalized in April 2004 when experts from several organizations (National Organization on Fetal Alcohol Syndrome, National Institutes of Health, Center for Disease Control and Prevention, and Substance Abuse and Mental Health Services Administration) developed a consensus definition of FASD [6]: "FASD is an umbrella term describing the range of effects that can occur in an individual whose mother drank alcohol during pregnancy," noting that, "These effects include physical, mental, behavioral, and (or) learning disabilities with possible lifelong implications".

Despite this wealth of evidence of alcohol's adverse effects, there are numerous challenges in obtaining an accurate and reliable diagnosis of FASD [7]. Characteristic facial abnormalities associated with FAS may not be present, or if present, can change with age. Environmental influences can impact physical, cognitive and behavioral problems. It is often difficult if not impossible to obtain an accurate history of maternal alcohol consumption. Moreover, independent of whether facial abnormalities are present, differentiation of FASD from other disorders with overlapping physical features and/or neurobehavioral deficits can be challenging. It is increasingly evident that new diagnostic tools that can provide sensitive biomarkers of prenatal alcohol exposure and that can be ethically applied would make a significant impact on the diagnosis and prevention of FASD. It is well established that early cognitive, educational, adaptive, and behavioral interventions can profoundly change the long-term outcomes and quality of life of these individuals and their families [8–10]. Knowing this impact, early screening tools are essential to help identify at-risk children at a young age and provide an objective clinical assessment that will allow these children access to early interventions and services (Fig. 28.1). In this Chapter, we discuss the evidence supporting the emerging potential for genetic, epigenetic, transcriptomic and proteomic approaches to elucidate further FASD

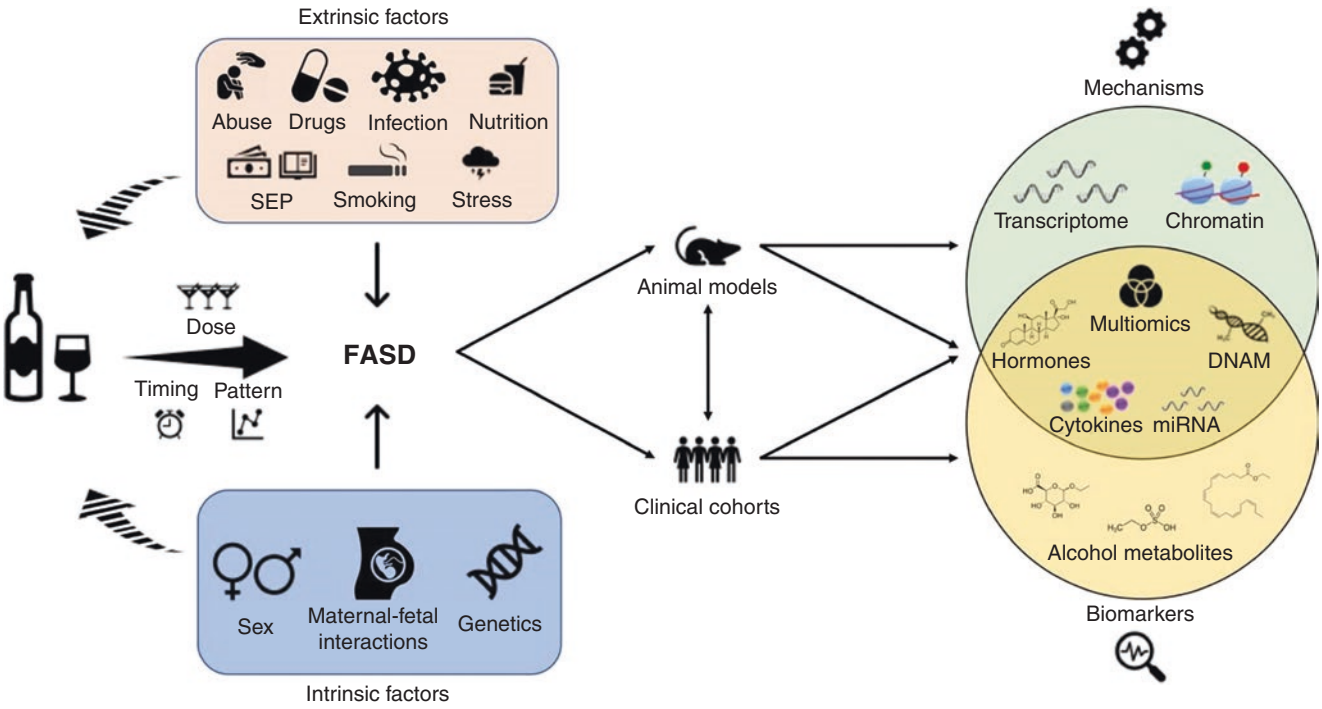

Fig. 28.1 An overarching framework of fetal alcohol spectrum disorder. Several factors influence the manifestation of fetal alcohol spectrum disorder (FASD), beginning with the pattern, timing, and dose of prenatal alcohol exposure. Extrinsic and intrinsic factors can also exert their influence at multiple stages, including both the prenatal period and postnatal development. These factors are studied in two basic approaches, animal models and clinical cohorts. Both approaches provide insight into the mechanisms driving the effects of alcohol on development and aid in the development of biomarkers of FASD. Finally, some types of analyses may be better suited than others for mechanistic insights or the development of biomarkers, although many show a broad range of applicability. *DNAm* DNA methylation; *SEP* Socioeconomic position

etiology, and to serve as potential biomarkers or signatures of early-life events, including prenatal alcohol exposure. We provide an overview of FASD and a brief history of the development of diagnostic criteria, review risk and resilience factors that impact the expression of the disorder, discuss genetic and epigenetic factors in FASD, and conclude by relating these findings to the clinical context.

FASD as a Common Neurodevelopmental Disorder: Prevalence and Diagnosis

Until recently, the prevalence of FASD was estimated at approximately 10 per 1000 children, using clinic-based studies or studies of individual communities [11]. However, it is now known that this is likely an underestimate of the true prevalence. Many children with FASD remain undiagnosed or are misdiagnosed; among other reasons, this might occur when trained dysmorphologists are not available to make a diagnosis or when individuals present with neurobehavioral deficits in the absence of dysmorphic features, and maternal drinking history is unknown. A recent study utilizing active-case ascertainment and a cross-sectional design to assess first graders at four community sites reported an estimated prevalence of FASD ranging from 1.1% to 5.0% [11]. While these findings may not be generalizable to all communities, these data suggest that the prevalence of FASD is likely higher than previously thought. Moreover, the prevalence of FASD is significantly higher than that of other common disorders, including Autism Spectrum Disorder, estimated at 2.20%–3.31% in 2016 [12], and Down Syndrome, estimated at approximately 1 in 1499 (or 0.67%) in the USA [13]. The relatively high prevalence of FASD highlights the urgent need for better recognition, diagnosis, and treatment strategies.

A variety of diagnostic guidelines have been developed over the years to assist clinicians in recognizing and diagnosing children exposed to alcohol *in utero* (reviewed in [7]). Following the identification of FAS, the term suspected fetal alcohol effects (FAE) [14] was introduced to describe the partial expression of FAS, where some but not all of the features seen in FAS are present, and recognizing that a range of deficits can occur, even in the absence of obvious facial dysmorphology. In 1996, an Institute of Medicine (IOM) committee identified four alcohol-related clinical diagnostic categories [15]: (1) Fetal alcohol syndrome (FAS: evidence of characteristic craniofacial dysmorphology, prenatal and postnatal growth restriction, and CNS neurodevelopmental deficits); (2) Partial fetal alcohol syndrome (pFAS: some but not all of the characteristics features of FAS and confirmed maternal alcohol exposure); (3) Alcohol-related neurodevelopmental disorder (ARND: evidence of CNS neurodevelopmental abnormalities and (or) of a complex pattern of behavioral or cognitive abnormalities, with confirmed maternal alcohol exposure); and (4) alcohol-related birth defects (ARBD: One or more congenital anomalies and confirmed maternal alcohol exposure). Categories 3 and 4 are not mutually exclusive. To "operationalize" the IOM criteria, Astley and Clarren [16] developed the FASD 4-digit diagnostic code, utilizing a Likert scale (1 = absence of the feature, 4 = strong presentation of the feature) to reflect the magnitude of expression of each of growth deficiency, the FAS facial phenotype, CNS dysfunction, and gestational exposure to alcohol. Updated versions of this system are now widely used.

Updated clinical guidelines for FASD have been published over the last 10–15 years, in general building on the IOM criteria and 4-digit code, and include those by Hoyme and colleagues [17], and two sets of Canadian Guidelines [18, 19]. Of note, the 2016 revision of the Canadian Guidelines made FASD a diagnostic term and collapsed the diagnostic categories to two: (*i*) FASD with sentinel facial features and evidence of impairment in 3 or more identified neurodevelopmental domains, with prenatal exposure to alcohol either confirmed or unknown and (*ii*) FASD without sentinel facial features, with evidence of impairment in 3 or more identified neurodevelopmental domains, and confirmed prenatal exposure to alcohol. These Guidelines also specify a category called "At risk for neurodevelopmental disorder and FASD, associated with prenatal alcohol exposure," which may help to identify individuals who are at risk. Most recently, the Diagnostic and Statistical Manual (DSM), fifth edition, from the American Psychiatric Association, includes "neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE)" (American Psychiatric Association 2013) under the "Conditions for Further Study" and also given as an example under "Other Specified Neurodevelopmental Disorder (315.8)". ND-PAE allows clinical assessment that encompasses the neurodevelopmental and mental health issues associated with prenatal alcohol exposure [20]; it can be diagnosed in either the presence or absence of physical effects of prenatal alcohol exposure, requires confirmed gestational exposure to alcohol, and includes symptoms in three broad domains—neurocognitive functioning, self-regulation, and adaptive functioning—that adversely impact the quality of life. The inclusion of FASD in the DSM is a critical step forward in both bringing FASD to the attention of clinicians and increasing access to services for individuals exposed to alcohol *in utero*.

Risk and Protective Factors for FASD: What Is the Evidence?

Following the identification of FAS, research in both human and animal studies blossomed, providing definitive evidence linking prenatal exposure to alcohol with FAS. Initially, this

link was interpreted in a fairly straightforward manner, as reflected in the first Surgeon General's warning (U.S. Surgeon General 1981, p. 9): "The Surgeon General advises women who are pregnant (or considering pregnancy) not to drink alcoholic beverages and to be aware of the alcoholic content of foods and drugs." However, no specific guidelines were proposed, as outcomes were highly variable, and little was understood of the relationship between the level of alcohol intake and the severity of the outcome.

In an attempt to understand both risk and protective factors, researchers initially focused on the assessment of quantity, frequency, pattern, timing, and duration of alcohol consumption. Studies have consistently found a positive correlation between maternal blood alcohol concentration and developmental alterations, including physical, physiological, neurobiological, cognitive, and behavioral deficits [21–24]. However, the greatest risk is associated with heavy episodic or binge drinking, which results in the highest blood alcohol levels [25–27]. In that regard, it became apparent that measures of average consumption levels (*e.g.*, average drinks per day or per month) may not be useful and in fact may be misleading. Rather, assessment of the number of drinks per occasion, or measures such as maximum drinks per occasion or number of "binges" are more relevant for understanding fetal exposure and risk in humans. The critical roles of timing and duration of exposure to risk are also well documented [28]. Organs and systems in the embryo are particularly vulnerable to structural and/or functional abnormalities during their period of most rapid growth and development. Two of the most vulnerable periods for brain development, for example, are the first half of the first trimester and the brain growth spurt that occurs in the third trimester [29]. Nevertheless, prenatal alcohol exposure can impair brain development during all stages of gestation, including effects on neurogenesis, differentiation, and synaptogenesis [28–31], as the brain develops over the entire gestational period and into postnatal life. Moreover, data suggest that variables such as quantity, frequency, pattern, timing, and duration of alcohol consumption cannot fully explain fetal or child outcomes. Increasing evidence suggests that numerous biological and environmental variables can further exacerbate risk or act as protective factors [10, 15, 25–27].

Maternal and Environmental Factors

Body profile, age, gravidity, parity, metabolism. The maternal body profile is associated with birth outcomes: lower than average height, weight and BMI are more often seen in women who have children with FASD than in those whose children are unaffected [26]. This may reflect undernutrition in these women, or possibly, that some of these women themselves may have FASD. The average risk of having an affected child and/or a more severely affected child is also increased in women who are older and who have higher gravidity (more pregnancies) and parity (more children) compared with the risk for younger women who drink at similar levels. Women who have children with FASD also have more miscarriages and stillbirths [25, 26]. The reason for these associations is not entirely clear, but may be related to the severity of addiction, such that these women cannot decrease their alcohol intake during pregnancy. Continued drinking over time may also cause deterioration of health and nutritional status. In particular, May and colleagues [32] reported that early age of initiating regular drinking may exacerbate the adverse effects of alcohol by increasing the amount of time that alcohol can affect biological and physiological processes important for alcohol metabolism. These effects can vary from one individual to the next and within individuals depending on nutrition, body weight, and physiological factors, as well as genetic and environmental influences [26].

Nutrition. Nutritional deficiencies has been linked to increased risk for adverse birth outcomes in general [25]. Importantly, alcohol intake can directly affect nutrient intake, resulting in primary malnutrition or undernutrition [33]. Because of its high energy value (7.1 kcal/g), alcohol may displace other food in the diet; as calories provided by alcohol are not associated with essential nutrients, intake of these "empty" calories can result in nutritional deficiencies. This is particularly problematic during pregnancy and lactation when nutrient requirements are considerably (10–30%) higher than in non-pregnant/non-lactating females. Economic issues may also play a role if a portion of the food budget is spent on alcohol in place of nutritious foods.

Secondary undernutrition is also an issue [33, 34], as the deleterious effects of alcohol occur in the placenta and at virtually every level of the gastrointestinal tract, resulting in altered metabolism, transport, utilization, activation, and storage of most essential nutrients. For example, effects on the umbilical circulation and on blood flow to the placenta will reduce oxygen supply as well as transport of essential nutrients to the fetus. The most commonly reported nutritional deficiencies and their major effects are shown in Table 28.1 [33–35].

Socioeconomic Position (SEP). SEP is a major determinant of health that encompasses a broad range of social and economic factors, such as income, education, occupation level, etc., that can represent health inequalities or influence an individual's health outcomes [36]. Of note, SEP is used here instead of the commonly used 'socioeconomic status' (SES), as the latter does not distinguish between actual resources and the status or rank-related characteristics of socioeconomic factors [36]. Although children with FASD have been identified in all SEP groups, data from several countries and epidemiological studies suggest that FASD may be more frequent in individuals in lower SEP categories

Table 28.1 Nutritional deficiencies and related FASD effects

Nutrients	Major effects
Protein/amino acids	Decreased organ growth and development, brain damage
Thiamin [vitamin B1]	Cardiovascular and nervous system function
Riboflavin [vitamin B2]	Activation of anti-oxidant enzymes
Vitamins B6 and B12	Cellular function, neurotransmitter synthesis, metabolism of glucose, lipids, proteins, alcohol
Vitamin E	Anti-oxidant, stability of free radicals
Selenium	Cofactor for antioxidant enzymes, thyroid hormones
Vitamin A	Organogenesis, cell and neuronal growth and differentiation
Vitamin C	Anti-oxidant
Folic acid	Premature birth, fetal malformations [eye, palate, GI tract, kidneys, skeleton, nervous system (including neural tube defects)]
Vitamin D and zinc	DNA, RNA stability, preterm delivery, increased incidence of birth defects
Choline	alcohol-induced alterations of the hippocampus and prefrontal cortex (PFC)
DH3 [Omega-3 fatty acids]	antioxidant mechanisms in the brain and liver

[27, 37]. For example, the quantity of alcohol consumed prior to knowledge of pregnancy, Total Distress score and SEP, taken together, were more highly associated with the diagnosis of an FASD than the quantity of alcohol consumed [38]. Associations between low SEP and FASD likely stem from findings that lower SEP may be associated with poor living conditions, poor nutrition, lower levels of education and employment, and high levels of stress, which are all associated with poorer birth outcomes. On average, infants born to women in lower SEP conditions have lower birth weight and length, smaller heads, more malformations, and higher levels of attention deficit disorder, whether alcohol-exposed or not [27, 37]. Studies also suggest that the severity of FASD effects is influenced or modulated by the stability and nurturing of the postnatal environment, which is also associated with SEP and maternal education, as well as marital and employment status [25].

Stress. Stress can be defined as a state of threatened homeostasis or an internal steady state [39]. Stressors, both physical and psychological, can disturb homeostasis and activate a set of adaptive responses that enable the individual to respond to and cope with the stressors and thus restore homeostasis. The two key components of the stress system are the autonomic nervous system that initiates a rapid "fight or flight" response, and the hypothalamic-pituitary-adrenal (HPA) system that initiates a slower and longer lasting hormonal response. Here we will focus primarily on the HPA axis due to its extensive and long-lasting effects on the body as well as its relevance for FASD. The HPA axis comprises a cascade of responses, ultimately resulting in the release of the stress hormone cortisol, which affects virtually every system in the body and facilitates coping and the restoration of homeostasis. In the short term, HPA activation is helpful, mobilizing energy, increasing cardiovascular tone and circulating glucose levels, and suppressing responses not immediately necessary for coping, including digestion and the immune response. In the long-term, however, chronic stress and chronically high levels of HPA activity/hormones, will have adverse consequences including fatigue, hypertension, ulceration, metabolic alterations, vulnerability to infections or diseases, and even neuron death.

Not surprisingly, exposure to stressors during pregnancy can have adverse effects on pregnancy outcomes, maternal and fetal health, and offspring's behavioral, immune, cognitive, and physiological development [40, 41]. Maternal stress has a marked negative impact on maternal endocrine and immune systems, which interact with each other and with the central nervous system in an intimate bidirectional manner. Alterations in the activity and function of any one of these systems will affect the others, and insults that alter the activity of these systems in the mother will affect the development of fetal metabolic, physiological, endocrine, and immune function, with potential long-term consequences for development and health.

Importantly, alcohol consumption during pregnancy can disrupt the normal hormonal interactions between the pregnant female and fetal systems, altering the normal hormone balance, including the activity of the HPA axis. Compounding the effect of prenatal alcohol exposure, children with FASD often experience a high level of early life adversity (*e.g.*, maltreatment, early caregiving disruption and contact with the foster care system, poverty, and familial adversity) [10, 42, 43]. The adverse effects of early life adversity can parallel in some ways the adverse effects of prenatal alcohol exposure, particularly on physiological and behavioral outcomes. In the human situation, however, it is difficult if not impossible to separate the effects of prenatal alcohol exposure from those of early life adversity, and studies evaluating the impact of prenatal alcohol may, at least in some cases, be studying both prenatal alcohol and environmental stress/adversity and/or their interactions.

Paternal Factors

In contrast to the large body of research on the influence of maternal factors on offspring outcome, much less attention has been paid to the possible role of preconceptional paternal factors. However, data indicate that a large proportion of women who drink alcohol associate with men who also drink alcohol [44]. Therefore, it is possible that at least some of the abnormalities attributed to the teratogenic effects of maternal drinking may be related to or exacerbated by paternal drinking. While evidence for the importance of paternal factors has emerged, the mechanisms responsible are not yet well

understood [25, 44, 45]. As alcohol contains toxins that can impact health, for men who use alcohol and father children, their semen may contain toxins that could damage the DNA of the fetus [45]. Moreover, both epidemiological and laboratory studies have shown genetic and epigenetic alterations that may underlie offspring outcomes following both maternal and paternal alcohol intake [46].

Genetics of FASD

Although FASD is essentially an environmental disorder, twin studies have shown that genetic mechanisms may play a role in resilience or vulnerability to the effects of alcohol exposure *in utero*. In one study, for example, while identical twins were 100% concordant for a specific FASD diagnosis, fraternal twins showed 56% concordance [47]. Of these, four pairs of fraternal twins had divergent diagnoses—partial FAS versus neurobehavioral disorder/alcohol-exposed—despite sharing 50% of their genetic information and presumably receiving virtually identical alcohol exposure. By contrast, full siblings who also share ~50% of their genomes, but may have different levels of alcohol exposure, show only 41% diagnosis concordance. These findings suggest that while fetal genetics likely play a key role in mediating the effects of alcohol exposure during embryonic development, environmental factors are also likely involved.

Here, we examine the underlying genetic mechanisms that may predispose and further exacerbate the effects of alcohol in animal models and humans, alike. To this end, we will discuss gene networks that may be involved in the development of the FASD sentinel facial features and potential comorbidities. Of note, the majority of these pathways have been identified through work in animal models, including but not limited to *Xenopus*, zebrafish, chicks, and mice, as these models allow for direct manipulation of genetic pathways and controlled alcohol exposure.

Genes and Cell Signaling Pathways Involved in FASD

Numerous pathways and signaling cascades have been associated with the development of physical alterations reminiscent of the FASD sentinel features, including both genetic pathways (*i.e.* bone morphogenetic protein (BMP), fibroblast growth factor (FGF), sonic hedgehog (SHH), Wingless (WNT) and biochemical factors (*i.e.* folic acid, retinoic acid, hormones, etc.). In particular, these signaling molecules are crucial for the proper craniofacial formation and mediate the genetic cross-talk necessary to form appropriate gradients and signaling pathway cascades during development. Furthermore, animal models have shown that genes within these networks and biochemical factors are altered by developmental alcohol exposure, potentially mediating the effects of alcohol on the developing organism, as outlined below. Importantly, both the direct influences of prenatal alcohol exposure on the expression of these genes or genetic variation within these pathways could lead to more or less vulnerability to the effects of alcohol.

SHH Mutations and Signaling Pathway Impairments in FASD

Sonic hedgehog (SHH) mutations are most commonly associated with craniofacial midline defects, such as holoprosencephaly and impairments in the frontonasal prominence and both maxillary and mandibular processes [48]. These craniofacial malformations are reminiscent of alcohol-induced craniofacial malformations across different species [49–51]. Importantly, it is known that alcohol activates direct antagonists of SHH signaling, such as the cAMP pathway and protein kinase A [52]. Furthermore, SHH and its respective pathways can be indirectly affected by anti-factor developmental morphogens such as retinoic acid, FGF, BMP4, and transforming growth factor β-1 (TGFβ-1) family member genes, which disrupt SHH gradients in the primitive streak and developing neural floor plate during gastrulation [53].

Of note, biochemical cross-talk between the cholesterol and SHH pathways may contribute to craniofacial malformations through SHH signaling. Indeed, the SHH protein requires the addition of cholesterol and palmitate to become biologically active [54]. Without these modifications, the SHH protein cannot be transported out of the cell or bind to lipid rafts within the plasma membrane for transport and signaling transduction. In addition, cholesterol can bind to the *Smo* protein directly; *Smo* is an SHH ligand required for activation of the GliA pathway and target gene activation for proper midline formation [55]. Given that these pathways are also affected by alcohol exposure (see below), SHH signaling may act as a key integration pathway for the sentinel features of FASD.

WNT Mutations and Signaling Pathway Impairments in FASD

Wingless (WNT) signaling is implicated in many developmental processes, including proper mitogenic stimulation, as well as cell fate specification and differentiation [56]. Studies across different model organisms have shown that alcohol can impact the WNT pathway at multiple points, causing aberrant cell migration and cellular differentiation in the gastrulating embryo (reviewed in [57]). Alcohol can also indirectly trigger the WNT pathway, causing abnormal expression of gene targets required for differentiation, migration, and proliferation and ultimately leading to neural crest cell apoptosis [58]. Importantly, acute alcohol exposure can impair the WNT/β-catenin canonical signaling pathway, causing

impairment of cartilage and bone formation, and neural crest cell lineage tissues [59]. Furthermore, disruption of cranial neural crest cell migration due to aberrant WNT signaling can result in impaired fusion of the nasal and maxillary processes, leading to cleft lip and/or cleft palate formation, which are the same craniofacial regions where key FASD sentinel features are found [60]. Taken together, this body of work indicates that the WNT pathway is a target of alcohol and may play a role in the development of FASD and its sentinel facial features.

FGF Signaling Pathway

Fibroblast growth factor (FGF) signaling is one of the most important signaling factors in the developing embryo, required for cellular proliferation, differentiation and migration, as well as proper axial development and craniofacial formation, specifically for ossification of cranial bones and suture homeostasis [61]. Given that several genes within this pathway are downregulated by prenatal alcohol in animal models, including *FGF2*, *FGF8,* and *FGFR2,* perturbation of this crucial craniofacial development pathway by alcohol could potentially drive some of the sentinel features observed in FASD [52, 62]. Of note, the FGF pathway also shows extensive crosstalk with the SHH, WNT, and retinoic acid pathways to control differentiation and patterning during development, suggesting that it may act as a focal point for genetic contributions to FASD [63].

BMP Signaling Pathway

Bone morphogenic protein (BMP) signaling is an important signaling factor in the developing embryo, required for cellular growth, differentiation and apoptosis. Similar to features seen in individuals with FASD, knock-out of BMP receptors impairs mesoderm and neural crest cell lineages, leading to lip, palate, and eye defects, as well as brain, cardiac, skeletal, and tooth defects [64]. Studies in both mouse models and clinical cohorts [65, 66] have shown that alcohol exposure during gastrulation causes dysregulation of the BMP signaling cascade, leading to congenital heart defects. As such, it is possible that alterations in the BMP pathway may play a key role in mediating cardiac-related deficits in FASD.

Retinoic Acid Deficiency and Signaling Pathway Impairments in FASD

Retinoic acid, the metabolized form of Vitamin A (retinol), is a crucial factor in the development, playing key roles in craniofacial, cardiac, and limb development. Importantly, retinoic acid deficiency at early gastrulation has been shown to produce FASD-like craniofacial defects such as smaller eyes, microcephaly, reduced axial development, and a lack of the forebrain ventricles [67, 68]. Furthermore, retinoic acid supplementation can partially rescue the effects of acute alcohol exposure [67, 69]. Of note, acetaldehyde competes with retinaldehyde for aldehyde dehydrogenases, becoming a rate-limiting step of retinoic acid metabolism. As such, variation in these genes could impact the bioavailability of retinoic acid, leading to deleterious effects on the developing organism. Taken together, these data suggest that retinoic acid or Vitamin A deficiency may be an underlying factor in the etiology of FASD, further exacerbating the developmental effects of prenatal alcohol exposure during early development.

Susceptibility and Resilience Genes and Factors in FASD

Although research has revealed many of the cell signaling pathways impacted by alcohol during development, insights into genetic factors that may further predispose an individual to FASD are not well understood. Various maternal factors act to increase the risk of alcohol's deleterious effects in the developing fetus. In particular, the presence of genetic polymorphisms of alcohol-metabolizing enzymes may increase or decrease alcohol's deleterious effects. For example, maternal polymorphisms manifesting as increased alcohol dehydrogenase activity and enhanced alcohol metabolism have been associated with a decreased incidence of alcohol teratogenicity [70, 71], possibly by impacting the capacity of the maternal-fetal unit to metabolize alcohol. As the capacity for alcohol metabolism among pregnant women can vary up to eightfold (from 0.0025 to 0.02 g/dl/h), the variation in phenotypic presentation of FASD in women consuming similar doses of alcohol could be mediated, at least partly, by genetic mechanisms.

Co-Morbidities with Other Developmental Genetic Disorders

DiGeorge syndrome, also known as 22q11.2 deletion syndrome (22q11.2DS), is a sporadic autosomal dominant disorder caused by a 1.5–3 Mb microdeletion on chromosome 22 encompassing approximately 30 genes [72]. Interestingly, 22q11.2DS shares many craniofacial and cardiac malformations with FASD, CHARGE syndrome, and retinoic acid embryopathy, including long philtrum, thin upper lip, upward slanted palpebral fissures, and aortic arch abnormalities [73, 74]. CHARGE syndrome refers to a set of developmental malformations that vary in their presentation, and include ocular coloboma (C), heart disease (H), choanal atresia (A), retarded growth and/or anomalies of the central nervous system (R), genito-urinary defects and/ or hypogonadism (G), and ear anomalies and/or deafness (E) [75]. In a recent study, mutations in the CHD7 (Chromatin helicase DNA binding

protein 7) gene, an evolutionarily conserved protein required for proper chromatin remodeling, were found in 83% of CHARGE syndrome patients [76]. CHARGE syndrome and 22q11.2DS brain aberrations such as reduced white matter tract volume and behavioral abnormalities such as attention deficit hyperactivity disorder (ADHD) are found in individuals with FASD and Autism Spectrum Disorder [76–79]. It is not a coincidence that these disorders share these malformations, as the malformations result from altered differentiation, proliferation, and migration of neural crest cells (NCC), which result in the hindbrain, frontonasal prominence, and pharyngeal arch aberrations. Interestingly, Sulik et al. found that alcohol exposure in a mouse model at embryonic day 8.5 causes craniofacial malformations reminiscent of DiGeorge syndrome and VeloCardioFacial (Shprintzen) syndrome: micrognathia, low-set ears, abnormal pinnae, a short philtrum, midline clefts in the nose, cleft palate, and ocular hypertelorism [80]. T-Box Transcription Factor 1 (*TBX1*, a gene deleted within the 22q11 region) heterozygous mice can also phenocopy the craniofacial malformations found in DiGeorge Syndrome and in FASD [81]. Finally, while the outcomes are more severe, retinoic acid embryopathy and Vitamin A deficiency syndrome produce craniofacial and brain malformations and upper trunk anomalies (heart, lungs, thymus, thyroid, for example) reminiscent of those seen in FASD as well as other syndromes including DiGeorge and CHARGE syndrome.

Of note, FASD sentinel facial features can vary as they reflect outcomes of craniofacial development specific to the time of alcohol exposure. As developmental processes have exquisite temporal regulation, timing of the alcohol insult will differentially affect the head, forebrain, and craniofacial development, contributing to the FASD spectrum. Nevertheless, the studies discussed above highlight the fact that genetic underpinnings of characteristic features of FASD suggest a powerful tool for further exploration of FASD etiology. Taken together, hemizygous expression of neural crest cell (NCC) genes such as *TBX1* could provide researchers with an opportunity to use 22q11.2DS as a model to study NCC-related aberrations that occur in FASD. Moreover, harnessing whole genome sequencing, mutations and epigenetic modulation of modifier genes can be utilized to understand better the variable expressivity associated with 22q11.2DS and other NCC-related disorders, including the role of NCC alterations in disorders such as FASD and CHARGE syndrome. Future studies will allow a better understanding of the role of developmental signaling pathways (SHH, FGF, BMP, retinoic acid) in FASD. Studies on the developmental signaling pathway defects shared with other disorders will expedite the development of pharmacological and other interventions that might help to attenuate or ameliorate the malformations and co-morbidities associated with FASD and other disorders.

Epigenetics of FASD

Although the exact molecular mechanisms underlying the effects of prenatal alcohol exposure on neurobiological systems are not yet fully elucidated, epigenetic mechanisms are prime candidates for the programming effects of environmental factors on physiological and neurobiological systems, as they may bridge environmental stimuli and neurodevelopmental outcomes to influence health and behavior well into adulthood. Epigenetics refers to modifications of DNA and/or its regulatory factors, including chromatin and non-coding RNA, that alter the accessibility of DNA to modulate gene expression and cellular functions without changes to underlying genomic sequences [82]. Patterns of epigenetic modifications, in general, have been closely associated with cell fate specification and differentiation, suggesting a crucial role for epigenetics in the regulation of cellular functions [83]. For a detailed overview of studies of DNA modifications and developmental alcohol exposure, please refer to [84].

DNA Methylation

DNA methylation is perhaps the most studied epigenetic modification and involves the covalent attachment of a methyl group to the 5′ position of cytosine, typically occurring at cytosine-guanine dinucleotide (CpG) sites. In addition to its association with gene expression, it also plays a key role in the regulation of developmental programs [85]. DNA methylation is also emerging as a potential biomarker for early-life exposures due to its stability over time and malleability in response to environmental cues [86]. Numerous studies have identified changes in DNA modifications in response to prenatal alcohol exposure, ranging from “bulk” (or total) levels to candidate gene approaches and genome-wide associations. Early findings from animal models demonstrated that prenatal alcohol exposure appears to impair the establishment of DNA methylation levels primarily at the bulk level, suggestive of broader reprogramming of downstream cellular and biological functions. However, more recent studies have shown that rather than a global decrease in DNA methylation, some genomic regions show specific directions of effect [87]. These studies have attempted to identify specific genetic loci that may be more sensitive to alcohol-induced epigenetic alteration, using both hypothesis and discovery-driven approaches. As well, these studies have focused mainly on genes with known functions related to the deficits observed in FASD (immune, stress, cognition, etc.), identifying more specific changes to DNA methylation patterns. However, they have not been as successful in pinpointing new targets and mechanisms in the etiology of FASD. As such, current approaches are beginning to move beyond tar-

geted analyses to assess the genome-wide effects of prenatal alcohol on the epigenome, without *a priori* hypotheses about which genes may be influenced. These approaches have yielded important insights into the broader molecular pathways involved in FASD pathogenesis, identifying new pathways and targets of alcohol-induced epigenetic alterations. Of note, recent studies have also used DNA methylation patterns as a potential biomarker of prenatal alcohol exposure. In animal models, our recent study showed that some DNA methylation patterns are concordant between the brain and blood of alcohol-exposed animals, suggesting that blood (white blood cells) may be an important surrogate tissue for the development of better FASD biomarkers [88], with critical implications for human studies. In humans, epigenetic biomarkers show promise for early screening of at-risk individuals, as the DNA methylome retains a lasting signature of gestational alcohol exposure in both the central nervous system and peripheral tissues (reviewed in [84]). Recently, DNA methylation profiles in a large cohort of children with FASD have established that epigenetic markers of prenatal alcohol exposure and FASD might exist and could have clinical utility [89]; importantly, these prenatal alcohol/FASD signatures were validated in a second cohort [90]. These findings set the stage for broader applications of DNA methylation in the context of FASD, creating a framework upon which to build future epigenomic studies of FASD and the development of biomarkers and assessment tools.

Chromatin Modifications

One step above DNA methylation lies the proteins—histones—that compact DNA in the nucleus [91]. Histones regulate access to the genetic material and gene expression patterns, which can be modulated through small chemical additions known as histone modifications. Importantly, these modifications play various roles depending on their genomic context and location on the histone and mediate the crosstalk between different levels of epigenetic regulation and cellular functions. In contrast to DNA methylation, the effects of alcohol on chromatin remain relatively understudied. Nevertheless, multiple lines of evidence suggest that developmental alcohol exposure can impact both the abundance and localization of different chromatin proteins and modifications. Several studies from animal models have shown differences in the levels of bulk histone modifications, generally revealing states less permissive to gene expression following prenatal alcohol exposure (*i.e.* genes turned off) (reviewed in [84]). A more recent study in the post-mortem brains of individuals with FASD showed similar patterns of repressive chromatin profiles when assaying bulk levels across a range of brain regions and ages [92]. In addition to bulk approaches, gene-specific and genome-wide histone modification profiles have also been characterized in cell culture and animal models, with widespread effects being identified across different models and the timing of exposures. As a whole, these results suggest that chromatin alterations are sensitive to the effect of alcohol and may play an important role in mediating the timing and dose-dependent effects of alcohol during development. In addition to histone modifications, several chromatin-associated proteins, such as MeCP2, can also regulate access to genes and modulate transcription factor recruitment to activate or repress gene expression. In particular, alterations to MeCP2 expression and regulation have been identified following alcohol exposure, suggestive of even broader alterations to chromatin profiles than suggested by altered histone modifications. It is also worth noting that mutations in MeCP2 lead to a severe neurodevelopmental disorder, Rett syndrome, suggesting that changes in its expression profiles could mediate some of the effects of alcohol [93].

Non-coding RNA

The final layer of epigenetic regulation is mediated through non-coding RNA (ncRNA), which performs a wide variety of regulatory functions in the cell. These transcripts play important roles in the regulation of messenger RNA and protein levels and are central to the integration of physiological and molecular cues necessary for brain development [94]. The atypical expression of many ncRNA in the brain has also been associated with various neurological disorders, including autism spectrum disorder, Fragile X, Rett syndrome, schizophrenia, and anxiety-like disorder [95]. The vast majority of research on the effects of alcohol on ncRNA has focused almost exclusively on micro RNA (miRNA), particularly with regards to alcohol-induced neurodegeneration and potential biomarkers of FASD (reviewed in [84]). Overall, these studies identified differentially expressed miRNAs with important roles in the development of deficits associated with prenatal alcohol exposure, including facial dysmorphisms, neuro-apoptosis, and altered neurodevelopment. Of note, the best characterized and replicated miRNA is miR-9, which displays altered expression patterns across multiple models and ages of alcohol exposure. Given its role in brain development and adult neural function, it may play an integral part in mediating some of the long-term deficits in FASD. Several miRNA have also been associated with prenatal alcohol later in adulthood, suggesting that they may retain a lasting signature of alcohol exposure and potentially act as biomarkers. A recent study followed up on this approach, showing that a panel of miRNA from the plasma of pregnant women who had consumed alcohol could predict infant neurodevelopmental outcomes on the Bayley scales of infant development at 6 and 12 months [96]. Although pre-

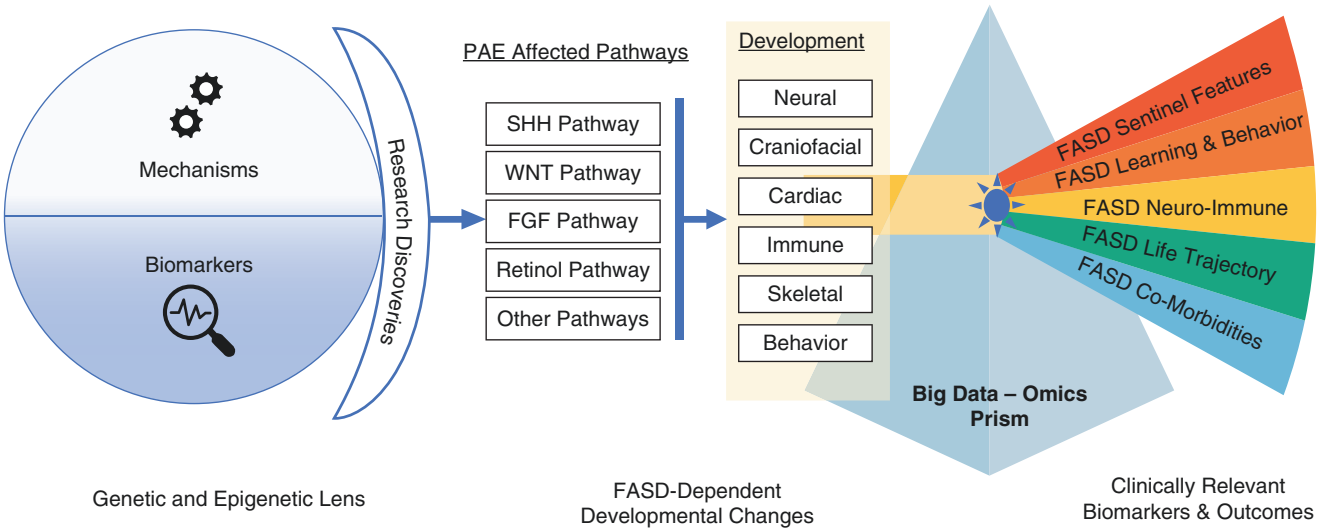

Fig. 28.2 Schematic of genetic and developmental pathways and clinically relevant outcomes in FASD. Genetics and epigenetics play a large role in susceptibility and resilience in individuals prenatally exposed to alcohol. Several genetic pathways appear to be involved in the downstream effects of prenatal alcohol exposure (PAE) on development. The resulting FASD-relevant impairments to neural, craniofacial, cardiac, immune, skeletal, and behavioral systems lead to a deeper understanding of the molecular mechanisms that drive FASD-dependent developmental changes. Moreover, integration and analysis of large new "omics" data sets hold much promise to identify clinically relevant biomarkers and outcomes from infancy into adulthood that can provide a basis for early intervention and perhaps for discrimination among FASD outcomes, life trajectories, and FASD co-morbidities

liminary, these findings suggest that ncRNA could act as viable biomarkers of alcohol exposure, while also pointing to the biological underlying pathways influenced by alcohol; these latter findings could have important implications for the development of novel therapeutic interventions in FASD.

Considerations for Epigenetic Research on FASD

It is important to note that the vast majority of studies on epigenetic mechanisms have been performed in animal models and primarily in samples from male subjects, highlighting the need for sex-specific investigations of prenatal alcohol effects as well as replication of these findings using clinical cohorts of individuals with FASD. Furthermore, given that epigenetic modifications are sensitive to environmental effects, it is crucial to replicate these findings in additional studies and take into consideration other risk and resilience factors such as SEP, nutrition, and genetic background when identifying new biomarkers. Several epigenome-wide analyses have shown associations of many of these factors with DNA methylation patterns, which could potentially overlap with those identified in FASD, given that SEP, nutrition, and genetics can influence the manifestation of FASD. Finally, because epigenetics essentially provides an interface between genes and environment, changes in epigenetic marks will not only capture those that are biomarkers of prenatal alcohol exposure, but also those that are biomarkers of the many other social, economic, environmental, and nutritional risk factors for FASD. This will allow for new research directions that examine the social epigenetics underlying FASD outcomes. Future studies will likely take these into account to create more robust associations and biomarkers of prenatal alcohol exposure (Fig. 28.2).

Transcriptomic and Proteomic Alterations of FASD

Transcriptomic Alterations

Although epigenetic factors are an attractive potential mechanism to mediate the biological embedding of early life events, their association with transcription makes gene expression programs a powerful target for assessing the molecular underpinning of alcohol-induced deficits. In particular, genome-wide investigations of gene expression programs have identified widespread alterations to gene expression levels in fetal, neonatal, and adult rodent models, providing important insight into potential mechanisms and pathways involved in alcohol-induced deficits. Given the importance of spatiotemporal gene expression during developmental patterning, it is perhaps not surprising that many of the alcohol-induced alterations to the transcriptome are closely related to the stage of development that was assessed [97]. For example, genes differentially expressed during gestation were generally associated with functions related to

cellular patterning, growth, and development, suggesting that alcohol can interfere with typical developmental programs. As gene expression is highly dynamic, quickly responding to environmental and cellular inputs, transcriptional alterations measured soon after alcohol exposure may reflect the intracellular response to the teratogen, rather than stable programming effects of prenatal alcohol on the genome. By contrast, gene expression profiling in the adult brain, long after the removal of alcohol, may provide additional insight into the long-term effects of prenatal alcohol exposure on cellular programs. Although these latter effects are usually subtler, long-lasting changes to the transcriptome have been identified in the brains of adult rodents, suggesting that alcohol can have lasting effects on the neural transcriptome [98, 99].

By contrast, alterations identified in the entire embryo or brain likely reflect the systemic effects of alcohol on the organism or CNS, respectively, and may reflect the broader effects of alcohol on biological functions. In particular, meta-analyses of gene expression patterns across multiple studies, ranging from whole embryos on embryonic day 9 in mice to the rat hippocampus on postnatal day 100, identified a general inhibition of transcription by alcohol, regardless of the model [97]. The differentially expressed genes identified in the combined analyses were mainly involved in protein synthesis, mRNA splicing, and chromatin function, suggesting that alcohol may broadly influence the regulatory systems of the cell, irrespective of the timing and dosage of alcohol exposure. More recent studies are beginning to focus on specific brain regions, providing insight into some of the functional deficits observed following prenatal alcohol exposure. For instance, gene expression patterns in the adult (postnatal day 70) mouse hippocampus are altered by a third-trimester equivalent (~ postnatal days 1–10, the period of the brain growth spurt in rodents) exposure to binge levels of alcohol, which may potentially be related to some of the spatial learning and memory impairment associated with alcohol [100]. A recent study also profiled gene expression patterns in human fetal cortical tissue from late first-trimester fetuses exposed to alcohol [101]. These embryos displayed a shift in the typical balance of splicing isoforms in addition to widespread alterations to transcriptomic programs, suggesting that alcohol may influence the fine balance of splice variants in the brain. However, these findings must be interpreted cautiously due to the small sample size ($n = 2$). Nevertheless,, taken together, these findings support the suggestion that gestational alcohol exposure can have both transient and persistent effects on the genome, which may influence the cellular response to alcohol and mediate the long-term deficits associated with FASD. Furthermore, alcohol-induced deficits may potentially arise through the disruption of epigenetic programs, concurrent with alterations to gene expression patterns.

Proteomic Alterations

Despite the valuable information gained from transcriptomic studies on the identity and level of gene expression altered by alcohol, changes in gene expression are often not directly correlated with changes in the amount of protein translated. While proteomic studies have generally been used to assess possible biomarkers of prenatal alcohol exposure in maternal-fetal interaction, including amniotic fluid and the uterine environment [102, 103], there is also interest in studying proteomics in mammalian animal models during early development to elucidate the molecular pathways affected by prenatal alcohol exposure [104].

Treatment of dams with a high dose of alcohol on gestation day 8, mimicking a binge exposure, resulted in reduced amniotic fluid levels of alpha-fetoprotein (AFP) compared to levels in control dams [104], suggesting that AFP could potentially be used as a biomarker for alcohol exposure. Importantly, this proteomic analysis of amniotic fluid on gestation day 17 (one day prior to birth) could discriminate between alcohol-exposed and unexposed embryos for high-risk dysmorphogenesis [104]. Similarly, proteomic analysis of placentas of rats exposed to alcohol from gestation day 5–19 (mimicking a more chronic exposure) identified 45 significantly altered placental proteins between alcohol-exposed and control placentas: proteins involved in alcohol metabolism (alcohol dehydrogenases and aldehyde dehydrogenases), as well as genes involved in cellular function, immune function, nutrition, fetal and neurodevelopment, and implantation was upregulated in the alcohol-exposed cohort compared to pair-fed controls [102]. Finally, human placenta proteomic profiles obtained from alcohol-exposed and non-exposed pregnancies demonstrated significant reductions in placental expression of VEGFR2 and annexin-A4, both of which could serve as potential placental biomarkers for prenatal alcohol exposure [103]. Thus, proteomic profiling is a tool which could be used to identify novel biomarkers of FASD, while providing insight into its possible molecular underpinnings.

Applications to Clinical Setting and Conclusions

FASD is the most common cause of neurodevelopmental impairments in the western world, with an estimated prevalence as high as 1.1–5%, affecting as many as 700,000 Canadians [11]. While prenatal alcohol exposure is the cause of FASD, not all alcohol-exposed individuals develop obvious deficits associated with FASD. In addition, determining if prenatal alcohol exposure has occurred is often very difficult due to the often subjective nature of self-reports of alcohol consumption or the unavailability of the birth mother

when assessing the child. Yet systematic reviews and meta-analyses have indicated that the global prevalence of alcohol use during pregnancy is 9.8% and it is estimated that 1 in 67 women who consume alcohol during pregnancy will deliver a child with FASD [105]. As we begin to understand the growing complexity of prenatal alcohol exposure and the co-morbidity of risk factors like nutrition, socioeconomic position and stress—and all of these in the context of an individual's own genetic variation and epigenetic responses—it is clear that there is no known safe level of alcohol consumption that can be ascertained or generalized from the data available to date. Furthermore, given that one alcohol-sensitive developmental window occurs before a woman may know she is pregnant and that over half of the pregnancies in North America are unplanned, even the most important educational warning of "Don't drink if you are pregnant" will not be sufficiently effective. Innovative and integrative public education approaches are needed to allow couples in their reproductive years to understand better and more fully the risk of alcohol consumption during pregnancy and possible FASD outcomes.

New diagnostic tools that can provide sensitive biomarkers of prenatal alcohol exposure and that can be ethically applied would make a significant impact in the diagnosis and prevention of FASD. The current diagnostic process for FASD is comprehensive but extremely time-consuming and costly, requiring a team of medical, psychological, educational and social specialists. Note, however, that instruments are currently being developed that may help to expedite identification of individuals who might have been exposed to alcohol prenatally [106]. This could increase the ability to identify those who might be at risk for FASD, and these individuals could then be referred for the full team-based assessment, which would expedite diagnosis. Although some children with FAS can be identified in infancy, most FASD diagnoses occur much later once children are in school or even into adolescence and adulthood, and a large number of children on the spectrum may never receive a diagnosis, particularly if they don't have the dysmorphic facial features of FAS [18]. That said, it is well established that early cognitive, educational, adaptive, and behavioral interventions can profoundly change the long-term outcomes and quality of life of these individuals and their families [8, 10]. Knowing this impact, early screening tools are essential to help identify at-risk children at a young age and provide an objective clinical assessment that will allow these children access to early interventions. Ensuring access to care for all children with FASD is a legislated right in Manitoba, as embodied by Jordan's Principle. Interestingly, the new ND-PAE diagnostic term ("neurobehavioral disorder associated with prenatal alcohol exposure") in the recent Diagnostic and Statistical Manual, fifth edition may be the exact key that facilitates early clinical evaluation with a recognized diagnostic assessment that allows access to early interventions. New medical guidelines and education programs are needed to incorporate such changes into a new standard health policy. The health care and education systems would need to be considered together under a broader framework to make this work. Importantly, we must also work to de-stigmatize FASD, identifying it as a neurodevelopmental disorder rather than blaming or shaming the mother, thus removing the judgement and stigma that often surround mental health and addiction issues.

Identifying inexpensive and reliable biomarker(s) for prenatal alcohol exposure and associated FASD outcomes will lead to more accurate and earlier identification of those at greatest risk, particularly the vast majority of children with FASD who are currently invisible to FASD diagnosis. The presence of meconium fatty acid ethyl esters (FAEE) in newborn infants has been shown to highly correlate with alcohol exposure [107]. However, meconium FAEE can only capture heavy drinking in late pregnancy, which is often already known in the person's history, and may have little predictive value for FASD outcomes. The present review highlights the emerging potential for genetic, epigenetic, transcriptomic and proteomic approaches not only to elucidate FASD etiology, but also to serve as potential biomarkers or signatures of early-life events, including prenatal alcohol exposure.

In this age of "big data" and larger child and adult cohorts, new frameworks on how to integrate genetic and epigenetic data with those of the many recent omics studies will not only identify new clinically relevant biomarkers of FASD, but will help elucidate the underlying molecular etiology of FASD and the many FASD comorbidities and related neurodevelopmental disorders, as well as the molecular etiologies that clinically distinguish them. New frameworks will also point the way to potential new treatments/interventions for children with FASD and other neurodevelopmental disorders.

Review Questions

1) Which of the following is the most common (highest prevalence?) neurodevelopmental disorder?
   a) Autism Spectrum Disorder
   b) FASD
   c) ADHD
   d) Cerebral Palsy
2) Most children with FASD remain undiagnosed or are misdiagnosed.
   a) True
   b) False
3) Which of the following is an FASD Diagnosis, according to the recent Diagnostic and Statistical Manual, 5th Edition.
   a) FASD with sentinel facial features and evidence of impairment in 3 or more identified neurodevelopmental domains, *with prenatal exposure to alcohol either confirmed or unknown*

b) FASD without sentinel facial features, with evidence of impairment in 3 or more identified neurodevelopmental domains, *and confirmed prenatal exposure to alcohol*
c) At risk for neurodevelopmental disorder and FASD, *associated with prenatal alcohol exposure*
d) All of the above

4) What is the recommended safe level of alcohol consumption during pregnancy?
a) 1–2 units of alcohol per week
b) 1–2 units of alcohol per week, but only in the first trimester
c) 1–2 units per week, but never together on the same occasion
d) There is no known safe level of alcohol consumption during pregnancy

5) Women with one FASD child have a higher risk of the next child having FASD.
a) True
b) False

6) Nutritional factors are risk factors for FASD because:
a) Alcohol is "empty" calories that may displace other food in the diet
b) Alcohol may exacerbate specific nutritional deficiencies during pregnancy
c) Deleterious effects of alcohol in the placenta and the gastrointestinal tract
d) All of the above

7) Which of the following is NOT a risk factor for FASD?
a) Socioeconomic Position
b) Chronic Stress
c) Paternal consumption of alcohol
d) None, all are risk factors

8) Which of the following statements regarding stress is incorrect?
a) Maternal stress affects the hypothalamic–pituitary–adrenal (HPA) system resulting in long lasting effects on the body
b) Maternal stress has a marked negative impact on maternal endocrine and immune systems
c) The stress effects of prenatal alcohol exposure are distinct from those of early life adversity
d) Stress can be defined as a state of threatened homeostasis or internal steady state

9) Twin studies of FASD provide the best evidence that genetics plays an important role in FASD because:
a) Identical twins are a 100% concordant for a specific FASD diagnosis
b) Fraternal twins show only a 56% concordance
c) A and B
d) A not B
e) B not A

10) Which of the following genetic pathways are considered to be affected by prenatal alcohol exposure during early development (select all that apply)?
a) Sonic Hedgehog (SHH)
b) Calcitriol (Vitamin D)
c) Fibroblast Growth Factor (FGF)
d) T-Box Factor (TBX)
e) Bone Morphogenic Protein (BMP)

11) Sonic hedgehog (SHH) mutations are most commonly associated with craniofacial midline defects, while bone morphogenic protein (BMP) signaling is an important signaling factor in the developing embryo required for cellular growth, differentiation, and apoptosis.
a) True
b) False

12) Genetic studies in model organisms often recapitulate developmental craniofacial and brain seen in FASD children. Other FASD developmental outcomes often less appreciated include (choose all that apply):
a) Congenital heart malformation
b) Significant increased incidence of miscarriage
c) Eye defects
d) Dental defects
e) Hearing defects
f) Kidney and liver defects

13) Genetic polymorphisms of alcohol metabolizing enzymes or in the genes and cell signaling pathways involved in FASD may identify susceptibility and resilience genes and factors of FASD.
a) True
b) False

14) FASD comorbidities with other developmental genetic disorder include (choose all that apply):
a) Attention Deficit Hyperactivity Disorder (ADHD)
b) Autism Spectrum Disorder (ASD)
c) Cerebral Palsy (CP)
d) DiGeorge syndrome
e) Charge Syndrome

15) Epigenetics refers to modifications of DNA and/or its regulatory factors that include (choose all that apply):
a) DNA Methylation
b) Changes in the DNA code
c) Chromatin/Histone Modification
d) Changes in Non-coding RNA expression

16) Because epigenetics essentially is the interface between genes and environment, changes in epigenetic marks will not only capture those that are biomarkers of prenatal alcohol exposure, but those that are also biomarkers of the many other social, economic, and nutritional risk factors for FASD.
a) True
b) False

17) Epigenetic modifications that tightly associate with transcriptional changes in gene expression can provide important insight into potential biomarkers and molecular mechanisms involved in alcohol-induced deficits.
    a) True
    b) False
18) Protein biomarkers of prenatal alcohol exposure have included:
    a) Reduced amniotic fluid levels of alpha fetoprotein (AFP)
    b) Significant reductions in placental expression of VEGFR2 and annexin-A4
    c) Presence of meconium fatty acid ethyl esters (FAEE) in newborn infants
    d) All of the above
19) Concerns regarding these biomarkers include (choose all that apply):
    a) Procedures can carry significant risk
    b) Access to tissue and reproducible results may be limited
    c) Indications of prenatal alcohol exposure are limited to late pregnancy
    d) Results may have limited predictive value for FASD outcomes
20) New frameworks on how to integrate genetic and epigenetic data with those of the many recent omics studies will not only identify new clinically relevant biomarkers of FASD but will help identify the underlying molecular etiology of FASD.
    a) True
    b) False

Answers

1b. 2a. 3d, 4d, 5a, 6d, 7d, 8c, 9c, 10a/c/e, 11a, 12all, 13a, 14a/b/d/e, 15a/c/d, 16a, 17a, 18a, 19a/b/c/d, 20a

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Fetal Alcohol Spectrum Disorder: Interventions

29

Jacqueline Pei, Aamena Kapasi, and Carmen Rasmussen

Learning Objectives

- Readers will understand the potential of interventions for children with FASD in supporting growth and healthy development of this population.
- Readers will be able to identify targeted intervention approaches for children with FASD.
- Readers will be able to identify integrated intervention approaches for children with FASD.
- Readers will understand the core components of successful intervention for children with FASD.

Highlights

- Both targeted and integrated intervention approaches have been shown to improve outcomes among children with FASD.
- Interventions should be built on existing strengths and address areas of challenge to promote healthy outcomes and improved quality of life, and not solely for the remediation of deficits.
- Five foundational components to FASD intervention are investing in relationships, adopting a strengths-based perspective, creating opportunities for expectation and engagement, recognizing FASD is a lifetime disability, and leveraging existing community capacities.

Introduction

Human beings are motivated to experience success and purpose in their lives, and individuals with Fetal Alcohol Spectrum Disorder (FASD) are no exception. In complex populations where the nature of the lifelong disability may be different for each individual, finding the pathway to success and purpose can be difficult. The goal of intervention approaches is to promote well-being and generate opportunities for meaningful and successful functioning, appropriate for the individual. Interventions are intentional change strategies designed with a goal in mind that matches what the individual is aiming to achieve. Clinicians and researchers intervene in the lives of clients in an attempt to influence change in a direction that is thought to be beneficial or informative. Health interventions are treatments, therapies, procedures, or actions that are implemented in response to health problems to improve conditions and achieve beneficial outcomes [1]. Interventions should be built on existing strengths and address areas of challenge to move towards healthy living and quality of life, and not solely for the remediation of deficits. Interventions must be implemented appropriately, in a timely and proactive manner, and may be tailored to the individual and their community. Importantly, communities of support and individuals with FASD should be engaged in developing intervention approaches to ensure that interventions are meaningful and feasible.

There is evidence supporting both direct and indirect intervention approaches to optimize growth opportunities for children and adolescents. Direct, or targeted, interventions are those that focus on specific skill growth and develop-

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ment. Indirect, or integrated, interventions offer shifts in environmental supports and expectations to foster broader goal achievement.

Over the past few decades, there has been an increased emphasis on evidence-based practice, which is the integration of research evidence, clinical expertise, and patient characteristics and preferences into the clinical decision-making process [2, 3]. According to an evidence-based practice approach, intervention choices should be informed by the best available research evidence [2].

The following chapter will describe both targeted and integrated evidence-based intervention approaches for individuals with FASD. We conclude with overarching themes of interventions for individuals with FASD, and key application considerations.

Targeted Interventions

Self-Regulation and Behaviour

The Alert Program® is a widely used program that focuses on self-regulation by teaching children to identify arousal states, choose appropriate strategies to help regulate identified arousal, and then problem-solve to select and implement a strategy to support optimal self-regulation [4]. The program is typically conducted in 12 sessions conducted weekly as children progress through three stages of the program: (1) identifying alert levels (2) experimenting with methods to change alert levels, and (3) regulating alert levels. One study found that children with FASD (6-11 yrs) in a modified Alert Program® treatment group showed improvements (compared to a control group) on a test of emotional problem solving and a parental report of executive functioning [5]. Another study [6] found that children with FASD (8–12 yrs) in an Alert Program® group displayed significant improvements in inhibitory control and social cognition, and parental report of behavior and emotional regulation, which were maintained 6 months later. Other studies have reported improvements in brain functioning and behavioural regulation [6] and increases in the frontal gray matter [7] among children with FASD following participation in the Alert Program®. The Alert Program® is currently being studied in a large randomized trial in Australia among school-age children with FASD [8] and was conducted in Canada among adolescents with FASD [9].

Another program, GoFAR, is used by interventionists to improve behavioural regulation and control, attention, and adaptive functioning among young children (aged 5–10) with FASD [10]. The program utilizes metacognitive techniques highlighting FAR: F (Focus), A (Act), and R (Reflect) and includes: (1) a computer game teaching FAR, (2) parental training in behavioural regulation, and (3) behavioural analog therapy sessions. Pilot studies conducted by Coles and colleagues have found that GoFAR leads to improvements in self-regulation skills [11], disruptive behaviour [10], and attention and adaptive functioning [12] among children with FASD. Importantly, children in GoFAR were able to learn the required skills and the program was well-received by both children and caregivers. Coles et al. [12] recommend future research on GoFAR with larger and more diverse samples.

Executive Functioning Abilities

Researchers have found that basic executive functioning (EF) capabilities, such as working memory and attention, can improve through a combination of direct training and appropriate strategy development and use. Caribbean Quest (CQ) is a computer game that facilitates EF development in children with neurodevelopmental difficulties through attention process training. This approach is based on the premise that neural plasticity or brain-based change, can be catalyzed through specific experience: if you train specific neural pathways, with sufficient duration, intensity, salience, and frequency you can strengthen that connection and improve function. In a combined group of children with FASD and Autism Spectrum Disorder (ASD), improvements were identified in working memory and attention following participating in CQ [13]. Gains were also noted in reading fluency suggesting some of the program benefits may have transferred to other academic areas.

Complementing the direct training, strategy development and use was supported during the CQ intervention. Developing and using strategies during the intervention proved to be an interesting and critical component. Specifically, children with FASD could increase the number of strategies they used to help support successful task completion—and their strategy use increased in spontaneity over time [14]. This is in keeping with a previous study in which researchers found that teaching children with FASD a simple rehearsal strategy for remembering information can improve their short-term memory [15].

Academics

One area that children with FASD have notable difficulty in is mathematics [16], and thus math skills have been an area of focus in FASD intervention research. The Mathematics Interactive Learning Experience (MILE) is an individualized math tutoring program developed by the same experts who developed GoFAR [17], geared towards improving mathematics difficulties among young children with FASD. MILE includes intensive, interactive, and individualized math

tutoring typically conducted in weekly sessions over ~6 weeks. The program supports underlying cognitive abilities involved in mathematics (e.g., working memory and visual-spatial skills) and uses manipulatives (e.g. physical objects) and special worksheets. MILE also uses the FAR technique noted above, with a goal to optimize retention and support translation to other practical domains such as behaviour. Application of this technique permits educators to work with children to understand their process of completing math tasks—'show me how you did this' not only to understand successful completion but also to observe the error process. In doing so educators are able to identify unique logic used by the children, and then use this knowledge to work with the children to develop alternative approaches.

In their initial study, Kable et al. [17] found that MILE led to significant improvements in math skills among children (aged 3–10) with FASD. Furthermore, even after a relatively short intervention with no follow-up sessions, the improvements were maintained 6 months later [18]. Coles, Kable et al. [10] have conducted further research on a community translation of the MILE program in order to reach more children with FASD. MILE was still efficacious in improving math skills when administered by community instructors over a 15-week period and the program was well-received and community instructors were very satisfied with their training [19]. Thus, MILE can be successfully translated and efficacious when conducted in community settings by community instructors, which allows for the program benefits to reach more children in need. The MILE program was replicated and extended in Canada [20] among young children (4–10 years) with FASD and/or prenatal alcohol exposure (PAE). Again, children participating in MILE improved more on math than those in an alternate intervention, and effects were maintained at a 6-month follow-up [20]. Finally, although mathematics has received considerable attention, other researchers [21] have shown that an intervention targeting language and literacy skills can also lead to improvements in these areas among children with FASD.

Activities of Daily Living

Activities of daily living include aspects of life that are essential to day-to-day functioning. Some of these activities include adaptive skills that gradually allow individuals to become independent and integrate into society. Social functioning is also a component of daily living which involves communication skills and the development and maintenance of relationships.

Adaptive functioning (e.g. hygiene,, safety knowledge, money concepts) is age dependent and characterized by performance. Often, individuals with FASD have difficulties with various daily living skills, functional communication, and community living skills [22]; however with appropriate strategies, adaptive skills can be improved. For example, in one study, fire and street safety was taught to children with FASD using computer games that employed virtual worlds, and a majority of children were able to learn the fire and safety skills and generalize them to a more "real world" situation [23].

Individuals with FASD often have difficulty with various aspects of social interactions [24], and these difficulties have been found to increase with age. An intervention based on the Children's Friendship Training Program has been adapted for children with FASD [25], which incorporates the use of parental assistance to help establish social networks and to practice social skills. Knowledge of social skills, demonstrated social skills, and behaviour significantly improved in children with FASD who received the intervention compared to those in a waitlist control group [26], and social skills continued to improve at a three-month follow-up. An intervention that taught social cognitive skills to target social communication issues has also been conducted as a case study with a child with FASD and demonstrated promising results [27]. Promising research has also emerged in the area of dog-assisted therapy [28]. A structured program that included six individual intervention sessions and six group activity sessions, which followed the Center of Dog Assisted Therapy model in combination with pharmacological treatment found improvements in social skills, externalizing symptoms, and FASD symptom severity [28].

Integrated Interventions

Integrated interventions are geared towards optimizing external systems of support. They may include the use of mentoring and coaching models to support individuals with FASD across a broad array of areas and they acknowledge the important impact family and professional supports have in assuaging difficulties and promoting well-being for individuals with FASD. Interventions which support parents and improve the parent-child relationship have been effective at preventing adverse outcomes in high-risk children and improving family functioning [29, 30].

Boost Parental Capacity

Mentorship models rely on a positive and trusting working relationship between caregivers and mentors as a catalyst for change. For example, the Coaching Families program uses mentors to provide caregivers of children with FASD with education about FASD, access to resources, and advocacy support [31]. A critical component of this program is establishing and maintaining strong relationships between the

mentors and caregivers whereby mentors encourage caregivers to identify and utilize self-care strategies, and work to help families cope and succeed in reducing family breakdown. After completing the Coaching Families program, caregivers reported a high level of satisfaction with the program, less stress, and a reduction in needs related to housing and transportation, family parenting, community development, and community resources [31].

The Families on Track program [32] is an integrated intervention that includes bimonthly in-home parent behavioural consultation with a weekly child skills group. The Families on Track program provides individualized and targeted behavioural consultation to families raising preschool and school-aged children with FASD who have clinically concerning behavioural problems [33], and this component of the program is based on the Families Moving Forward program [26]. The program uses motivational interviewing techniques and provides caregiver education, support, teaching skills, and links to community services and emphasizes a neurodevelopment viewpoint and positive behaviour support [33]. In comparison to a community care group, caregivers of children with FASD participating in Families Moving Forward reported greater met family needs, a greater sense of parenting efficacy, more parental self-care, and decreased child disruptive behaviour as well as a high level of satisfaction and treatment compliance [26, 33].

The second component of the Families on Track program is a weekly child skills group that is based on the pre-K/Kindergarten curriculum of the Promoting Alternative THinking Strategies (PATHS) program [34]. Children were taught skills including self-control, emotional understanding, positive self-esteem, peer relationships, and interpersonal problem-solving skills [32]. After the program, children with FASD demonstrated improvements on emotion regulation, self-esteem, and anxiety. Important caregiver outcomes including knowledge of FASD and advocacy, attributions of behaviour, use of antecedent strategies, parenting efficacy, family needs met, social support, and self-care demonstrated medium-to-large effects. Additionally, families reported high program satisfaction [32].

Nurture Parent-Child Relationships

Child-Parent Psychotherapy (CPP), is a relationship-focused, reflective, and developmentally attuned program for young children with histories of trauma, maltreatment, and associated problems with behaviour, self-regulation and posttraumatic stress [35]. The principle goals of CPP include: (1) creating safety in parent-child relationships and surrounding environments, (2) expanding parental responsiveness/attunement, (3) promoting the parental capacity to balance parent and child needs, and (4) modifying maladaptive perceptions [36]. The program allows for flexibility in administration based on the child's age, trauma history, and caregivers' functioning. CPP was combined with aspects of another program, Mindful Parenting Education, in a neurodevelopmentally informed clinical intervention for young children with FASD [37]. Mindful Parenting Education [38, 39] is a program that emphasizes reflection, empathy, compassion, and regulation. Parents in this intervention received psychoeducation aimed to improve their understanding of regulation challenges among children with FASD and reduce stress through improvement in their own regulation skills, parenting skills, and sense of self-efficacy. When combined with Child-Parent Psychotherapy with a sample of young children with FASD there was the improvement in child developmental functioning, including interpersonal, adaptive, motor, and cognitive domains, and parents' caregiving skills [37].

The capacity to manage emotions when children have self-regulatory difficulties can be a challenge for parents of children with FASD. Programs also use mindfulness strategies to promote positive self-regulation strategies in caregivers and their children, as seen in the Parents Under Pressure program [40]. Mindfulness strategies focus on the psychological process of bringing attention to the present moment and adopting a non-judgemental and accepting attitude. A feasibility study was conducted that provides preliminary support for an adapted version of the program with families with FASD. Parents reported qualitative improvements in their parent-child relationship, child's psychosocial distress, and increased understanding of the neurological basis for some of the challenging behaviours [41].

Increase Home Stability

A known risk-factor for adverse outcomes in many children, and particularly those with FASD, is multiple home placements and instability [42]. A project called Promising Practices for children and adolescents suspected or diagnosed with FASD in out-of-home care targets the issues of multiple home placements [43]. This program includes a commitment to children and youth with FASD having permanent placements, transitional plans, FASD assessments, respite care, collaborative support plans developed with caseworkers, foster care support workers and foster parents, training, and consistent caseworker-caregiver interactions [43]. In a pilot study, Promising Practices were associated with reduced risk behaviours and school absences and increased placement stability among youth with FASD [44]. Furthermore, project regions that received the enhanced practice standards had lower placement change than those that did not receive them, and worker contact was found to be predictive of placement stability [43].

Promote Mental Health and Well-being

Individuals with FASD have been identified as presenting with significant mental health needs [45]. Intervention efforts to address this need have included initiatives that target well-being and apply relational approaches to enhancing well-being; other programs are geared towards specifically responding to identified mental health needs.

Wellness needs have been targeted through mentorship programs such as the WRaP (Wellness, Resilience, and Partnership) program [46]. The WRaP program is situated in select Alberta schools, and provides strength-based supports to children and adolescents affected by FASD. WRaP mentors work one-on-one with individuals with FASD to guide and empower them in vocational, educational, and community supports, and be active members in society. The mentors also work with educators, community agencies, and other professionals to provide guidance and support in order to build the capacity of school personnel, develop community partnerships, and support student engagement, success, and social, emotional, and physical well-being. Researchers have found that genuine, safe, and compassionate relationships are key to facilitating the growth and development of skills.

Specific mental health needs in individuals with FASD can be supported through counselling and psychotherapy that is tailored towards the client and considers their FASD diagnosis in treatment [47]. A model of FASD-informed clinical practice describes three components to counselling individuals with FASD: reflection, communication, and action. Counsellors working with individuals with FASD are encouraged to participate in proactive reflective practice of the clinical process, in which they identify possible indicators of success, but then adjust and shift their indicators and their practices in response to observed outcomes—thereby tailoring their approach to unique client needs that are not always easy to anticipate. Multiple levels of communication are also key when counselling individuals with FASD, including between counsellors and other service providers, supervisory staff, and FASD consultants. Finally, counsellors must be active in their approach and use practical and pragmatic strategies when working with this population [47].

When working with adolescents with FASD, program staff describe success when they work with both parents and adolescents to teach them important psychoeducation and stress-coping skills. A program that targets addictions, called Project Step-Up, has found success using this approach [48]. Project Step-Up provides separate weekly groups for parents and adolescents with FASD to prevent and reduce alcohol use and focuses on alcohol education, fostering coping skills, and teaching adaptive responses to substance-related social pressure [48]. Parents are taught about the effects of prenatal alcohol exposure, as well as strategies for handling parenting challenges. Among adolescents, with FASD a significant decrease was found in light/moderate drinkers' self-reported alcohol risk and in alcohol-related negative behaviours compared to the control group. Importantly, adolescents who were absentee or infrequent drinkers did not increase in alcohol-related outcomes.

Support Healthy Families

Mentoring and coaching have been found to be efficacious in supporting parents with FASD. The Parent-Child Assistance Program (PCAP) targets mothers and parents through a three-year home visitation/harm reduction approach aimed to prevent alcohol-exposed births [49]. Case managers provide positive and empathic relationships, and they assist clients with problem-solving and referrals to community services [49]. The FASD adaptation provided specific FASD training for case managers and service providers working with *caregivers with FASD*. Overall, case managers helped parents with FASD increase their ability to access and the quality of services available to this population in the community [49]. Another similar supportive intervention is Step-by-Step for caregivers with FASD, which provides mentors to secure and strengthen community connections and support, income and employment support, and stable housing as needed [50]. Mentors also refer family members for neuropsychological assessments and provide addictions supports, crisis management, and recreation resources [50]. Mentors of this program shared that while advocacy and supports provision are important, much of a client's success is largely dependent on the stability in other domains of life, particularly in having their basic needs met consistently [50].

Plan for Activities of Daily Living (ADL) during Transitional Years

Although ADL's may be enhanced through direct initiatives with the individual, external systems of support are also important to support adaptive functioning. This is particularly true during transitional periods during the adolescent years. Areas of importance to consider in activities of daily living for adolescents with FASD include housing [51], employment [52], and parenting [53]. It is imperative that transition planning begins before the age of 18 to ensure supports are in place during the transition from adolescence to adulthood.

Adopting an Evidence-Based Intervention Framework

Research conducted to date has revealed that intervention efforts can have a positive impact on outcomes for individuals with FASD. However, implementation of specific intervention efforts has been slow; programs have not always been feasible in generalized practice as they may be unwieldy, community-specific, or require high levels of training or technology support. As such, knowledge translation efforts have included the extraction of consistent mechanisms within intervention efforts that appear to be core contributors to outcomes, and which can be readily applied or implemented within existing practice. This approach to intervention provides a framework through which evidence may be applied within existing strategies, community initiatives, and programs—ultimately moving towards an FASD-informed approach to service delivery. This framework provides five foundational components important to FASD intervention: investing in relationships, adopting a strengths-based perspective, creating opportunities for expectation and engagement, recognizing that FASD is a lifetime disability, and leveraging existing community capacities. Researchers examining intervention approaches have revealed that neither targeted nor integrated intervention programs need to be FASD-specific, but rather FASD-informed, in which those involved are aware of the brain-based origin of difficulties observed. Moreover, FASD-informed inventions that also incorporate a responsive trauma-informed philosophy may provide the highest level of proactive support for all individuals with complex needs. Embedded evidence-based practice with clearly defined goals, indicators, and outcomes is a core component of any responsive approach to permit the evolution of approach as dictated by emerging evidence.

Investing in Relationships

A core theme throughout much of the intervention research is the value of relationships. The investment in trusting relationships, with caregivers, families, and individuals affected by FASD is not only a core aspect of many intervention strategies but also a requisite component.

As such intentionality in our approach to relational service delivery is critical. This may include:

- Asking about healthy relationships in the child's life
- Identifying the role and responsibility of each core person in a child's life
- Emphasizing the need for mentors or coaches to help bridge transitions in the child's life and create capacity within new systems of support (e.g. from school to work settings)
- Seeking support that may facilitate the maintenance of existing natural supports
- Asserting the importance of collaborative relationships between professionals to promote continuity and stability of all service delivery
- Asserting the need to devote resources towards foundational relationships within any direct intervention model

Adopting a Strength-Based Perspective

Intervention efforts are typically geared towards addressing areas of difficulty and consequently improving functioning. This is in keeping with clinical efforts to refine our understanding of the deficits most commonly present in individuals with FASD. Yet researchers have asserted that overemphasis on individuals' psychological deficits, without equal focus on strengths and resources compromises a full understanding of the individual [54, 55]. Balancing the understanding of cognitive functioning, with the understanding of both areas of strength and impairment may therefore provide the ideal intervention framework by acknowledging and capitalizing on the strengths of individuals with FASD. Such an approach may ultimately lead to an enhancement of functioning rather than exclusively focusing on reducing or eliminating deficits [56] and also increase the engagement of all involved. To do so this may include:

- Identifying the child's strengths and areas of interest; balanced with areas of difficulty and need
- Creating conversation around experiences of success
- Reinforcing desired behaviour
- Scaffolding learning opportunities with clearly identified indicators of success
- Identifying clear goals that are focused on the desired outcome—rather than a problem to mitigate

Creating Opportunities for Expectation and Engagement

Recognizing that children and youth of all ages need to be active contributors to their intervention process is critical. This expectation of involvement creates the conditions for growth for all children. Although children with FASD face challenges in their development and their daily functioning, the majority will grow to be autonomous adults, and will not meet the criteria for guardianship or external decision-making governance. Consequently, setting gradual and developmentally appropriate expectations for involvement and decision-making set the framework for later life requirements. Moreover, engagement in this process often facilitates the implementations of embedded supports and strategy

use that are meaningful and therefore more readily generalizable for the individual. Across many direct intervention initiatives, strategy development and use were observed to be feasible, generalizable, and useful for children and youth with FASD—when they were active participants in the development of those strategies. Supporting caregivers and service providers in setting realistic expectations and engaging youth in intervention processes may include:

- Identification of key transition periods and proactively planning for both support and skill acquisition
- Asking the youth what supports are most useful to them
- Involving youth in meetings—and planning ahead for ways to make their involvement meaningful and suited to their unique skills and comfort levels
- Providing children and youth at all ages and stages with safe opportunities to make decisions and choices that are safe and meaningful

Recognizing FASD Is a Lifelong Disability

At any stage of development consideration for unique developmental presentation along with environmental demands will allow for the most effective individualized intervention and treatment planning that is proactive and anticipates the needs of the individual. Specific developmental considerations include:

- Transition periods: into school, middle/junior high, senior high, and adulthood
- Identifying appropriate expectations for autonomy: housing, employment, relationships and parenting demands

Leveraging Existing Community Capacities

Prenatal brain injury creates neurological vulnerabilities. Consequently, environmental factors such as stability, attachment, and consistent relationships carry greater potential for impact on the developing child—both harmful or promotive. Thus, investing in existing systems of support, to optimize ways in which service delivery may be FASD-informed in order to strengthen the environmental context and leverage the promotive potential is essential for this population. In this intentional fashion, both targeted and integrated supports may be woven together, between systems as required by the unique needs of the individual. Importantly, the navigation of such integrated systems requires the support of informed advocates and well-linked professionals.

Supporting these efforts will likely include:

- Enhancing caregiver supports to increase access to respite and in-home supports, and caregiver supports, to enhance family cohesion and stability and reduce the risk of burnout and family or placement breakdowns
- Training and education opportunities to support shared understandings for all professionals. Access to training that is specific to an area of specialization (e.g. education or justice) is ideal. (see CanFASD.ca for training available).
- Support navigating systems, which is critical for both families and individuals with FASD. Identification of an advocate or system navigator who may provide support by identifying and accessing needed support; and then linking supports to facilitate communication is ideal.

Conclusions

Interventions can have positive impacts on children and adolescents with FASD. Although many risks have been identified for individuals with FASD, by optimizing intervention initiatives we increase the likelihood of healthy outcomes. There are several evidence-based interventions that are efficacious for children and adolescents with FASD including interventions targeting specific skills (e.g., mathematics, EF) as well as more integrated interventions (e.g. family supports). These efforts have not only provided insight into the potential for intervention with this population but have also identified some core considerations for the implementation of FASD-informed supports. Moving forward more research is still needed in this area, particularly related to the long-term maintenance of intervention effects, transfer of improved skills to other domains, and key transition periods (including adolescence and early adulthood). As medical technology also continues to grow, many other intervention possibilities may begin to emerge. Additionally, continued evaluation of existing programs will provide further direction regarding effective approaches that are feasible and impactful. Intervention efforts can have positive impacts in terms of skill development and effective environmental support. Proactive planning, and effective team building, leadership, and collaboration create the necessary conditions for these optimized efforts. The field of FASD is still in its infancy and thus intervention wisdom is still emerging. There is every indication that with increasing knowledge, ongoing creativity, and continued efforts interventions and consequent outcomes for this population will continue to improve.

Multiple Choice Questions

1. Interventions to support math development in children with FASD have included: (**Answer—D**)
   a) Targeted interventions
   b) Use of the FAR technique
   c) Support of underlying EF skills
   d) All of the above
2. Evidence-based practice primarily entails: (**Answer—B**)
   a) Decision-making that is grounded in current research
   b) Integration of research evidence, clinical expertise, and patient characteristics and preferences into the clinical decision-making process
   c) Extensive applied training that exposes clinicians to a breadth of experience that drives clinical decision-making
   d) None of the above
3. Engaging youth with FASD in intervention planning may include: (**Answer—D**)
   a) Asking the youth what supports are most useful to them
   b) Involving youth in meetings—and planning ahead for ways to make their involvement meaningful and suited to their unique skills and comfort levels
   c) Providing children and youth at all ages and stages with safe opportunities to make decisions and choices that are safe and meaningful
   d) All of the above
4. Integrated interventions focus on: (**Answer—A**)
   a) Optimizing external systems of support
   b) A specific skill
   c) Self-regulation
   d) Parent–child relationships

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Part IV

Cerebral Palsy

Cerebral Palsy: Clinical Vignettes

30

Michael Shevell

Highlights

- Cerebral Palsy is phenotypically heterogeneous with respect to its motor, cognitive and behavioural characteristics.
- Children and families with cerebral palsy are often more challenged by the associated co-morbidities, such as epilepsy, than direct aspects of the disease.
- Cerebral Palsy is the end result of a vast and expanding array of acquired and genetic disorders.

Introduction

Two outstanding clinicians of the late nineteenth century, Sir William Osler and Sigmund Freud were the first to synthetize their clinical observations of motorically impaired children into the symptom complex they each termed "cerebral palsy" [1, 2]. During the twentieth century, much effort was expended through systematic study in elucidating identifiable risk factors, causal mechanisms, classification schemes, and basic interventional principles and approaches [3, 4]. Over the past generation, advances in imaging and molecular biology have deepened both our understanding of causation and offered new mechanisms of pathogenesis. This enriched understanding has opened new pathways for prevention and interventions that are not merely symptomatic in their effort but act as potential modifiers of underlying biologic processes [5, 6]. Furthermore, function, activity and participation, as conceptualized by the WHO ICF Model (International Classification of Functioning, Disability and Health), have been applied to individuals with cerebral palsy to go beyond a merely biological or medical approach [7, 8].

Cerebral palsy remains a clinically diagnosed entity. No single feature is pathognomonic and no laboratory finding is necessary for diagnosis. Its intrinsic variety has been well-captured in its most recent consensus definition that currently remains without serious challenge; "Cerebral palsy (CP) describes a group of permanent disorders of movement and posture, causing activity limitation, that are attributed to nonprogressive disturbances that occurred in the developing fetal or immature brain. The motor disorders of CP are often accompanied by disturbances of sensation, perception, cognition, communication, and behavior, by epilepsy, and by secondary musculoskeletal problems" [9].

This consensus definition puts forward that the *sine qua non* of cerebral palsy is an early neuromotor impairment which results from a static disturbance of the immature brain. This neuromotor impairment may take a variety of forms on the formal neurologic examination and may have a varying severity with reference to its functional effects. The static disturbance itself may be congenital or acquired and may arise antepartum, intra (or peri-) partum, or post partum beyond the newborn period. For many individuals with cerebral palsy, the core feature of neuromotor impairment is accompanied by one or more comorbidities. For many such affected individuals, it is one or more of these comorbidities that provides the greatest challenge to health and fuller participation in society.

The clinical vignettes that follow are meant to illustrate the diversity that is cerebral palsy. Where illustrative, key points in cerebral palsy will be mentioned in the brief accompanying case commentary. No doubt these key points will be explored in much further detail in the remaining chapters of this section. The final case vignette is meant to clarify from the case definition what is exclusionary, and hence not truly cerebral palsy.

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Case #1

Cory is the second born of fraternal twins. The twins were conceived by IVF with their mother and father as donor eggs and sperm. There is no known family history of any neurological or neuromuscular disorders. The parents are of Northern European heritage and deny consanguinity.

Mother was a 35-year-old primigravida who received regular antenatal care throughout and did not smoke, drink alcohol or use illicit drugs. Aside from longstanding hypothyroidism for which she had received synthyroid, she had no medical issues. The pregnancy itself was uneventful aside from twin gestation and antenatal ultrasounds did not detect any abnormalities in either twin.

Mother began to experience premature contractions at 30 weeks gestation that was treated with hospitalization, strict bed rest and salbutamol. Despite these measures, contractions persisted and her cervix began to dilate progressively. Prior to a cesarean section to deliver the twins, she received both betamethasone and magnesium sulfate. Her membranes did not rupture prior to delivery and she did not experience any peripartum fever.

Cory's fraternal twin sister was delivered first without incident. Cory too was delivered without incident. His APGARs were 6 and 8 at one and five minutes respectively. A cord pH was not drawn. He did not require any case room resuscitation efforts. His birth weight was 1.7 kg, close to the mean for his gestational age. He received surfactant by an endotracheal tube prior to transfer to the hospital's Level II-B NICU for observation. He initially breathed on his own without assistance, however over the first day of life respiratory difficulty became apparent with progressive hypoxemia and hypercapnia, necessitating CPAP and oxygen supplementation for three days. He never required intubation and ventilation.

His stay in the NICU was largely uneventful. Initially, by gavage, he was fed completely orally by two weeks of age. A cardiac murmur consistent with PDA was confirmed on cardiac echo. This responded well to a single dose of indomethacin. Routine cranial ultrasounds done prior to discharge did not document any intraventricular hemorrhage, ventricular dilatation or periventricular echogenicity. Cory was discharged home in the care of his parents at 7 weeks of age weighing 2.8 kg, fully breastfed and on no medication.

By around 2–3 months, Cory was noted to lag behind his twin sister in terms of motor milestones (rolling, sitting, crawling, pulling to stand and cruising). He first walked independently at 20 months. Fine motor, speech and social skills were comparable to his twin sister.

At age two, prompted by neuromotor delay, Cory was seen by a pediatric neurologist. The examination was entirely normal except for: tightness in both heel cords with a propensity to walk on tiptoes, a "W"-squat when sitting, brisk reflexes at the knees and ankles with spread, cross-adductor reflexes and upgoing plantar responses bilaterally. No upper limb involvement could be discerned. Cory was diagnosed as having a spastic diplegic variant of cerebral palsy. A 3 T MRI documented mild periventricular leukomalacia.

Cory was referred to physiotherapy for stretching exercises and the fitting of bilateral AFOs. Continuing to walk on his tiptoes and unable to jump or run with dexterity, he underwent lower extremity botoxulinum injections beginning at 3 years of age every 6 months until school age with some functional motor improvement. He was deemed not a candidate for tendon releases or selective dorsal rhizotomy due to his good GMFCS (Level II) status.

At age 5, Cory entered a regular school with his sister. At that time he was ambulating independently, but still had to grip the handrail to go up or down stairs. He was participating in community sporting activities. While school integration initially went well, by Grade 2 Cory was noticeably struggling. He was noted to be fidgety and restless with difficulty sitting still in the classroom. He would stay on task intermittently but was easily distractible, obviously impulsive and rarely completed assignments. A neuropsychological assessment undertook just prior to starting Grade 3 documented normal verbal and non-verbal intelligence, but significant attentional limitations and a slight lag in academic skills acquisition. A trial of stimulant medication was undertaken with excellent effect. He remained on a stimulant medication through the remainder of primary and secondary school. He graduated with his sister, entering subsequent to high school a college-level IT program.

Commentary-Case #1

Cory's case highlights prematurity as a risk factor for later cerebral palsy [10]. In high-resource settings, roughly 45% of cases of cerebral palsy arise in infants born prior to term (ie 37 weeks gestation), though these births comprise perhaps 10% of all births [11]. The risk of later cerebral palsy is inversely related to both gestational age and birth weight. The risk of later cerebral palsy, and other neurodevelopmental disorders, is appreciable enough to mandate heightened surveillance for developmental concerns for premature infants in a systematic and standardized manner in targeted neonatal follow-up clinics.

The characteristic underlying pathology for cerebral palsy in prematurely born infants is periventricular leukomalacia (PVL), representing the sequelae of an acquired ischemic injury preferentially affecting the deep subjacent white matter of descending motor tracts from the motor cortex (precentral gyrus) located immediately adjacent to the lateral

ventricles [12]. The lamination of these descending motor fibers is such that these fibers are those descending to provide motor control to the lower extremities. The PVL is best visualized on MR imaging by a thinning of the white matter with cortical gyri descending almost to the edge of the lateral ventricles, dilatation of the lateral ventricles with a blunting of their posterior configuration, and T2 white matter signal hyperintensity [13].

Clinically these individuals will manifest the spastic diplegic variant of cerebral palsy [14]. While diplegia strictly means that two extremities (without further specification) are involved, in this instance this inevitably refers to the lower extremities. Clinically this is evident by gross motor delay, a toe walking gait, a "W" pattern of sitting on the floor with the knees together and the ankles apart, tightness of the heel cords and a spectrum of pyramidal tract signs in the legs (increase tone in the gastrocnemius and hamstrings, hyperreflexia with spread, a cross adductor reflex, possible clonus, up going plantars). These findings may be asymmetric, reflecting an asymmetric pattern of white matter injury.

A number of factors are thought to predispose premature infant to experience PVL. These include; a narrow range of constant cerebral perfusion pressure as a function of mean arterial pressure (MAP) resulting in more instances of a pressure-passive cerebral circulation leading alternatively to ischemia (low MAP) or hemorrhage (high MAP), an anatomical watershed zone in the periventricular white matter between the distal ends of penetrating vessels, and a propensity to cardiorespiratory insufficiency and resulting ischemia attributable to the innate physiologic immaturity of cardiac and respiratory systems [15]. Of particular note is the emergence over the past decade of evidence for the particular susceptibility of the predominant oligodendroglial cell type between the 26 and 34 weeks of gestation (pre-oligodendrogial cells) to all matters of acquired ischemic injury [16]. Thus the pathogenesis of PVL is multifactorial.

Cory's case highlights that spastic diplegia for most is mild with ambulation achievable without assistance (GMFCS Level I or II) [17]. The major challenge is the neuromotor impairment with the mainstays of management physiotherapy, orthotics, botulinum toxin, and where indicated (not in Cory's case) orthopedic (tendon releases) or neurosurgical (selective dorsal rhizotomy) interventions.

For a subset of those with spastic diplegic cerebral palsy, the motor challenges of the toddler and preschool child may be replaced by school-age difficulties that may be cognitive (ie learning disabilities) or behavioural (ie ADHD) in origin. Indeed children with PVL are at high risk for such sequelae for which interventions are available to optimize the outcome, as in Cory's case.

Case #2

Melanie was the first child of her 16-year-old mother. The mother had concealed her pregnancy from her family, friends and healthcare providers. The pregnancy first came to attention when she presented to her local emergency room with a fever and a foul-smelling vaginal discharge of at least 3-days duration. Her cervix was already partially effaced and dilated. By dates and ultrasound, the fetus was estimated to be in the 24th week of gestation. Admitted to hospital, premature labour ensued leading to a cesarean section delivery the next day. Pathologic examination of the placenta was consistent with chorioamnionitis, the result of infection.

Melanie weighed but 520 grams at birth. Her APGARs were 5 and 6 at one and five minutes respectively. The cord pH was 7.2 with a bicarbonate of 18 and a base excess of −6. Respiratory distress was rapidly evident necessitating intubation, ventilation and oxygen supplementation for 10-weeks. Subsequent to extubation and cessation of ventilation, bronchopulmonary dysplasia became evident with oxygen dependency.

Melanie's in-hospital course was tumultuous. There were at least three episodes of sepsis necessitating fluids and antibiotic coverage, necrotizing enterocolitis requiring ischemic bowel resection and re-anastomosis, apneas and bradycardias of prematurity, anemia of prematurity, jaundice requiring both phototherapy and exchange transfusion, and a hemodynamically significant patent ductus arteriosus refractory to indomethacin which required intraoperative ligation for closure.

A cranial ultrasound on Melanie documented initially at one week of age a Grade III IVH with intraparenchymal extension. Subsequent ultrasounds revealed some post-hemorraghic hydrocephalus that stopped short of the need for drainage or shunting and the development of multiple periventricular cysts and echodensities.

At close to term corrected age, Melanie was discharged in the care of foster parents requiring home oxygen and diuretics for her BPD. These were all stopped at 6 months of age. Obvious signs of neuromotor impairment were evident in the first year of life and characterized by truncal hypotonia, appendicular stiffness and motor delay. She was formally diagnosed at 6 months of age with a spastic quadriparetic cerebral palsy variant affecting her lower than upper limbs. Over time obvious cognitive delay became evident with concomitant bulbar difficulties characterized by dysarthria and dysphagia. An MRI at 2 years of age documented dilated ventricles (hydrocephalus ex vacuo), periventricular cysts, triangular blunting of the posterior horns of the lateral ventricles, increased T2 signal intensity diffusely and thin white matter volumes.

Now 10 years of age, Melanie was never able to walk independently with or without assistance. She can use an electric wheelchair with adaptive seating. She attends a special school setting and has been unable to learn to read or write. She remains dependent on activities of daily living due to her motoric limitations. She communicates predominantly by pictograms and is beginning to use a Dynavox and computer. She has never experienced any evident seizures. She remains with her original foster parents who have now formally adopted her.

Commentary-Case #2

Melanie's case illustrates the opposite extreme in comparison to Case #1, of the consequences of prematurity as it relates to the sequelae of cerebral palsy. Born near the lower limits of viability, Melanie's birth was complicated by premature prolonged rupture of membranes (PPROM) that frequently results in a peripartum ascending infection (ie chorioamnionitis). The resulting release of a variety of pro-inflammatory mediators (eg cytokines) may both induce an acquired CNS injury and potentiate the deleterious effects of exposure of the immature brain to ischemia and hypoxia [18].

The innate friability of the extremely premature intracranial vasculature coupled with the occurrence of a MAP outside the quite narrow range that ensures a constant CPP (through sepsis, hypotension or cardiorespiratory instability) results in intraventricular hemorrhage, which, when severe may extend, intraparenchymally as occurred with Melanie. Intracranial extension combined with the preferential locus of ischemic injury to the periventricular white matter (see Commentary-Case #1) may lead, with tissue remodeling and reorganization, to the formation of periventricular cysts [14, 15, 19]. These cysts are recognizable in Cranial Ultrasound studies in the NICU and are a potent poor prognostic indicator for the affected infant [20].

The inevitable outcome of cystic periventricular cysts is what we observe in Melanie; severe spastic quadriparetic cerebral palsy with cognitive dysfunction that is indicative of widespread white matter injury. Bulbar dysfunction often co-occurs, representing aberrant descending cortical input, leading to dysphagia and dysarthria resulting in both feeding and speech difficulties. Symptomatic epilepsy, though not present in Melanie's case, frequently complicates matters and may be intractable, requiring a multitude of anti-convulsant medications to effect a modicum of seizure control.

Case #3

Esther was born at term to a 25-year-old primigravida. The mother denied any history of neurological problems in her extended family or that of the father. The pregnancy itself was entirely uneventful with regular antenatal care provided by a midwife and a normal routine ultrasound at 16 weeks gestation. Alcohol, tobacco and illicit drugs were not used and there was no gestational diabetes, per-vaginal bleeding, hypertension or inter-current maternal infections noted. Labor began spontaneously at 39 and 3/7 weeks gestation. Membranes ruptured shortly thereafter and the amniotic fluid was clear without meconium staining.

Delivery took place in a birthing centre located down the street from a tertiary general hospital with a high-risk delivery unit. Labor progressed smoothly with almost full dilatation (9 cm) within 8 h of commencement. However coincident with this, there was the loss of the fetal heart rate by auscultation, followed by pronounced vaginal hemorrhage and uterine tenderness suggestive of abruptio placentae. A rapid transfer to the adjacent hospital's obstetrical OR was undertaken. Within one hour Esther was delivered by cesarean section.

Esther was born flat without respiratory effort or heart rate. Case room resuscitation involved chest compression, intubation, epinephrine by the endotracheal tube, and fluid boluses. The cord pH was 6.76 with a bicarbonate of 10 and a base excess of −20. APGARs were recorded as 0, 1, 2, 4, 6 at one, five, ten, fifteen and 20 min.

While en route to the regional cooling centre located at a tertiary pediatric hospital, obvious seizures were noted and Esther was loaded on phenobarbital. Therapeutic hypothermia with total body cooling for 72 h was initiated 3 h after birth concomitant with continuous EEG monitoring. EEG monitoring revealed ongoing electrographic seizures that eventually responded to additional phenobarbital, midazolam, levetiracetam and topiramate.

The neonatal course was complicated by oliguria, hematuria and elevated creatinine. Liver transaminases were also elevated. Inotropic pressor agents (dobutamine and dopamine) were required. An absent suck and gag reflex were noted. This was followed by an inability to feed orally, prompting the use of gavage feeding for two weeks. MR imaging at 10 days of age confirmed severe ischemic changes involving the perirolandic cortex and deep gray matter structures (putamen and thalamus) bilaterally. Discharge was at 3 weeks of age on a combination of levetiracetam and topiramate.

Esther's first year of life was characterized by obvious signs of secondary microcephaly, neuromotor impairment

(profound gross motor delay, spastic limbs) and failure to thrive necessitating placement of a gastrostomy tube. At 6 months of age, she began to experience flexor infantile spasms for which vigabatrin provided some aspect of seizure control.

Now 17 years of age, Esther is unable to roll and has no functional hand use or evident language (receptive or expressive) skills. She is entirely fed by a gastrostomy tube and ambulates by others in a wheelchair. She has had multiple orthopedic surgical procedures including tendon releases, and bilateral hip surgery, and is awaiting surgery for her scoliosis. A baclofen pump was placed in late childhood to lessen overall spasticity, improve care (dressing, transfer, changing diapers) and relieve pain. Excessive drooling was initially treated by intra-salivary botox injections followed by selective salivary duct ligation. She has pronounced dystonic posturing of her upper limbs (for which she receives trihexyphenidyl) superimposed on diffuse asymmetric spasticity involving both the hemi-body and the upper and lower limbs. Audiometric and visual assessments have documented a severe bilateral sensorineural hearing loss and cortical visual impairment. She has continued to experience multiple seizure types and remains on four anticonvulsants for a modicum of seizure control. She remains at home with her mother as the parents divorced when she was 5. Mother never returned after her delivery to her prior work and career in order to provide care at home for Esther.

Commentary-Case #3

Esther's case is representative of the major intrapartum cause of cerebral palsy: birth asphyxia. Often wrongly identified as *the* major cause of cerebral palsy overall, in actuality, it is responsible for up to 20% of cases of cerebral palsy in high-resource settings and in half of these cases a concurrent predisposing factor (eg congenital anomaly, intra-uterine growth retardation, placental infection) is identified to also be present [21]. Birth asphyxia is also frequently over-diagnosed based on the reliance on notoriously non-specific single markers of potential perinatal adversity (eg meconium staining, APGAR scores) [22].

At present no single gold standard for the diagnosis of intrapartum birth asphyxia exists. Consensus criteria, largely based on the work of assembled Task Forces comprised of experts, have emerged [23, 24]. These include the essential presence of both a moderate to a severe grade of neonatal encephalopathy in the first few days of life and a markedly acidotic cord pH (pH <7.0, bicarbonate less than 16 and a base excess greater than −12). An intrapartum timing is suggested by documentation of a probable sentinel event, transition to a Category III fetal heart rate tracing during labour and delivery, APGAR scores less than 5 at 5 min, newborn neuroimaging findings consistent with antecedent acute asphyxial injury, and evidence of multi-organ failure. Furthermore, there must be no evidence for another evident cause and the eventual observed neurodevelopmental outcome must be consistent with asphyxia. This typically refers to either a spastic quadriparetic, dyskinetic or mixed cerebral palsy variant, but may on occasion feature other subtypes of cerebral palsy or other neurodevelopmental disorders such as global developmental delay, intellectual developmental disorder or an autistic spectrum disorder. The need for case room resuscitation efforts and EEG findings such as burst-suppression, background attenuation and multifocal epileptiform abnormalities, though not currently part of consensus criteria, can also be considered supportive of the occurrence of intrapartum asphyxia. It is important to note that at present this exercise in consensus criteria for the diagnosis of intrapartum asphyxia only applies to term infants.

In Esther's situation, the case details can support no etiologic supposition other than intrapartum asphyxia. The combination of newborn seizures and brainstem dysfunction implies a severe grade of neonatal encephalopathy. The cord gas is acidotic in all parameters reported, and a sentinel event is present, as is a Category III fetal heart rate tracing. The APGAR score is low at five minutes and beyond and Esther required case room resuscitation and multiple organ systems gave evidence for asphyxial injury. The imaging pattern documents what has been termed the 'acute near-total' variant of the term intrapartum asphyxia [25]. This pattern reflects an almost total interruption of fetal blood flow immediately preceding delivery lasting between 15 to 30 min in duration. Anything less than 15 min appears not to have neurologic sequelae, while a duration longer than 30 min is felt to be invariably fatal resulting in stillbirth. This pattern affects preferentially the high metabolic activity of the peri-rolandic cortex and deep gray matter structures of the thalamus and basal ganglia. This is in contradistinction to the 'prolonged partial' variant that evolves over hours, days or weeks of ischemic fetal perfusion and preferentially involves the parasagittal watershed areas of the brain [26]. The two variants may co-exist and indeed it is often inferred that the prolonged partial variant may predispose the fetus to a superimposed acute near-total event.

Not surprisingly infants with an acute near total asphyxia are at risk for a mixed cerebral palsy variant featuring both spastic quadriparesis and dyskinesia as occurred in Esther's situation [27]. The newborn MRI on the third or tenth day of life is a biomarker for this later risk, while the severity of the neonatal encephalopathy directly relates to the probability of such an adverse outcome [28]. While prospective studies have provided evidence for the potential beneficial modifying effects of cooling subsequent to term asphyxia, this benefit is not uniformly derived (NNT = 8) and is restricted to those with a moderate neonatal encephalopathy [29].

Esther's eventual cerebral palsy is at the highest functional level for gross motor impairment (GMFCS Level V) which affects roughly 15% of children with cerebral palsy [17]. Her experience of multiple co-morbidities is demonstrative of the range of potential consequences of severe neuromotor impairment and acquired brain injury; intractable epilepsy, developmental delay and intellectual disability swallowing and feeding difficulty, primary sensory impairments, pain and orthopedic deformities. These co-morbidities disproportionately affect and cluster amongst those with a severe level of functional motor impairment [30]. Interventions are possible in each to minimize their effects, ameliorate outcomes and improve an individual's and family's Quality of Life [31]. Not infrequently severe cerebral palsy will impact a family unit leading to separation and divorce. While one cannot directly medically address the injured brain, symptomatic treatment is available and can be targeted. Missing from Esther's case are difficulties in behavior and sleep disturbances that are frequently under-recognized in this clinical situation.

Case #4

Jason is the third-born child of his parents. He has two older sisters who are well. Both parents are also in good health and a family history of any neurological problems was denied. Mother's pregnancy with Jason was entirely uneventful. She received antenatal care throughout and did not experience any per-vaginal bleeding, gestational diabetes, hypertension or inter-current infections. She was not prescribed any medications and did not smoke, drink or use illicit drugs. She had two antenatal ultrasounds that were reported as normal. Labor itself was spontaneous with a vaginal vertex delivery without assistance or meconium staining occurring 4 h after labor began. Jason's birth weight was 3.5 kg and APGARs were 8 and 9 at one and five minutes. Aside from mild physiologic jaundice not requiring phototherapy, the neonatal course was uneventful and he was discharged home in the care of his parents at 3 days of age.

To the mother's recall, Jason always had a left-hand preference. She reported this to her pediatrician at the 6 month well visit and was told that this was likely because she herself was left-handed. At 9 months of age Jason had symptoms of an otitis media which prompted a visit to the after-hours walk-in clinic run by the pediatric group practice the family frequented. There the covering pediatrician noted that Jason only reached for toys with his left hand. When she questioned Jason's mother about this, the mother confirmed that this has always been so and attributed, as she had been told, to her being left-handed. Jason was uncooperative to a formal examination but the pediatrician did take note that his right hand was closed and fisted throughout most of the encounter. A referral was made to a pediatric neurologist.

Jason was seen by the neurologist just a few days after his first birthday. Developmental milestones were appropriate and he was noted to be socially engaging, curious and inquisitive. He continued to demonstrate a decided left-hand preference, but when the left arm was restrained he would try to reach without success with his right arm. The right hand was fisted but would on occasion open. The right hemi-body had a relative paucity of antigravity movements compared to the left and tone was increased over the right. These findings appeared to be more evident in the upper than lower extremities. Stretch reflexes were brisk in the right upper and lower extremity with spread apparent in the upper extremity and the plantar response was clearly up going on the right and down going on the left.

Arrangements were made for an EEG and an MRI/MRA study. The EEG showed widespread slowing over the left hemisphere. The MRI showed a central porencephalic cyst in the distribution of the left MCA with ipsilateral dilatation of the left lateral ventricle. Aside from a thin M1 segment of the left MCA, the MRA study was considered to be within normal limits.

At a follow-up visit with the neurologist two weeks after the imaging study, parents were informed of a formal diagnosis of a hemiplegic cerebral palsy variant attributed to a presumed perinatal arterial ischemic stroke (PPAIS). A detailed coagulation and cardiac workup were undertaken with negative results. A referral was made to both occupational therapy and physiotherapy.

Annual follow-up visits documented a stable right hemiplegic CP. Developmental trajectory, including speech and language skills, was normal. Jason was able to ambulate independently with just slightly less fluidity than that of typically developing children (GMFCS I) and he became independent with respect to all expected activities of daily living. For a time both an AFO and wrist splint were utilized. With the entry into regular school and an above-average performance in Grade 1 without behavioural difficulties, regular visits with the neurologist were discontinued at age 7.

Jason returned to see the neurologist at age 11 after two paroxysmal events over the preceding week. Both started with clonic jerking involving the right upper extremity, which progressed after 2 min to a loss of awareness and unresponsiveness lasting a further 3–5 min. There was no fall to the ground observed or any involvement of the right lower extremity or right side of the face noted. Fatigue and headache lasting a further 15 min followed both events. An EEG revealed an active right central epileptiform abnormality. Levetiracetam was chosen for seizure control however it proved ineffective. Subsequently, clobazam and then oxcarbazepine were added sequentially also without substantive effect.

At the age of 13, Jason was experiencing his characteristic seizures almost daily. On two occasions, when he had forgotten to take his medications (once while on vacation with his family, the other while sleeping over at a friend's house) he had experienced subsequent to the loss of awareness and unresponsiveness a five-minute generalized tonic-clonic seizure with tongue biting and urinary incontinence. Following hospital admission, featuring an extensive surgical evaluation and an inter-disciplinary seizure conference, Jason underwent resection of his zone of epileptogenic activity under intra-operative EEG guidance. Subsequent to this surgical intervention, he experienced no further seizures and indeed was weaned off all anti-convulsant medications within a year.

Jason would go on to graduate from university and find steady employment as a media buyer for an advertising agency. He learned to drive, enjoyed a variety of outdoor pursuits and by age thirty he was married and the father of one child.

Commentary-Case #4

Jason's case illustrates that about a third of children with cerebral palsy do not come from an at-risk group with evident antenatal, perinatal or post-natal risk factors [32]. These children are most frequently picked up by; the detection of motor delay in the first two years of life, the observation of a hand preference before the first birthday, or the occurrence of seizures [33]. In this instance, the finding of a porencephalic cyst on imaging at a year of age suggests a remote stroke for which epochal (ie antenatal, perinatal, postnatal) dating on the basis of the imaging finding is simply not possible. As the hand preference was not acquired, but rather always present, and there was no evident perinatal adversity or acute stroke symptomatology as a newborn (ie typically newborn seizures), one can presume that it occurred antenatally.

Jason's case also illustrates that though neuromotor impairment (in this case hemiplegia) is the *sine qua non* of cerebral palsy, for many affected individuals it is *not* the major healthcare burden. For Jason this would be his intractable epilepsy. Epilepsy occurs in up to half of all individuals with cerebral palsy and for a subset, the seizure disorder may be refractory to medical management [6]. In such instances, if appropriate medications in appropriate doses have been tried and there are functional limitations, surgical resection of the epileptic tissue may be possible, and be of enormous therapeutic benefit, if; this tissue is focally restricted and can be demonstrated to be the source of the individual's seizures, and its removal does not engender a greater functional deficit than that already existing.

Jason's narrative demonstrates that for many individuals the functional severity of their cerebral palsy is minimal and that participation in life is full (eg education, recreation, employment, marriage, and parenthood). Over half of individuals with cerebral palsy are at either Level I or II on the various standardized functional scales (GMFCS [gross motor], MACS [bimanual dexterity], CFCS [communication]) [17, 34]. Missing from Jason's case is the now evidence-based use of both TMS (transcranial magnetic stimulation) [35] and CIMT (constraint-induced movement therapy) [36] as modifiers of upper limb functional impairment. Indeed there is evidence that their combined use has a synergistic effect in improving hand function [37].

Case #5

Massimo is the youngest of two children of his parents. He has an older brother who is entirely well. Parents are of southern Italian heritage. While consanguinity was denied, their families are from the same village in Calabria. While both parents are in good health, on the father's side at least three family members have died in their forties from a GI malignancy.

Mother's pregnancy, labor and delivery with Massimo were entirely without incident. She had regular antenatal care and was without any complications such as per-vaginal bleeding, hypertension, gestational diabetes or inter-current infections. She was not prescribed any medications and did not smoke, consume alcohol or use illicit drugs. Labor was at 38 weeks gestation. Delivery was via a cesarean section for the breech presentation that did not respond to an attempt at an external version. Massimo's APGARs were 8 and 9 at one and five minutes respectively and his birthweight was 3.8 kg. Aside from physiologic jaundice requiring phototherapy, there were no neonatal difficulties and he was discharged home after five days.

Parents were first concerned about Massimo's motor development around his first birthday. He only began to sit when placed shortly after his first birthday, crawled at 15 months, and took his first steps at 18 months. At this point, his pediatrician undertook a CK and thyroid studies with negative results and referred Massimo to a pediatric neurologist for further assessment.

When seen at 2 years of age, Massimo was walking but had an unsteady gait with a propensity to fall at times. His reach was slightly dysmetric. His speech was clear and he demonstrated excellent comprehension. The tone was diffusely decreased, stretch reflexes were difficult to elicit (but present), and plantar responses were downgoing bilaterally. An MRI was undertaken within a month and this demonstrated slight atrophy of the superior portion of both cerebellar hemispheres and the vermis. A diagnosis of probable ataxic-hypotonic cerebral palsy was made, referral to both occupational therapy and physiotherapy were undertaken, and a follow-up visit was arranged for 6 months later.

Unfortunately, Massimo was unable to attend the follow-up visit as scheduled as he was hospitalized with pneumonia. When next seen by the neurologist at 3 years of age, the parents reported that he continued to be unsteady in his gait and to fall frequently. A note from his rehabilitation centre emphasized his therapists' concern about a worsening in his gait and fine motor skills over time. On formal examination, the neurologist was struck by some evident dysarthria, problems tracking objects with his eyes, a tendency to thrust his head to view things in the periphery (oculomotor apraxia), and drooling. There was more dysmetria on finger-nose testing, as well as truncal and gait ataxia than previously noted.

Based on the evident deterioration, a metabolic workup together with specific screening tests and a repeat imaging study were scheduled. The metabolic screening was negative, however, the repeat imaging study documented a greater degree of cerebellar atrophy than previous. Screening tests revealed low levels of immunoglobulins (IgA, IgG, IgM) and an elevated alpha-fetoprotein. This prompted sequencing of the ATM gene which revealed a homozygous known mutation, thus confirming the diagnosis of ataxia-telangiectasia.

Subsequently, Massimo developed both cutaneous and ocular telangiectasias in late childhood. He lost the ability to ambulate by age 10 becoming wheelchair-bound. Progressive swallowing difficulties led to the placement of a gastrostomy tube at age 12. In his teens, he developed both dystonia and chorea. At age 16 he was diagnosed with Hodgkin's Lymphoma that proved refractory to treatment with vinblastine, adriamycin and methotrexate leading to his death just prior to his eighteenth birthday.

Commentary-Case #5

Massimo's case is illustrative of what are collectively known as "cerebral palsy mimics". These are disorders that upon initial presentation may be mistaken for cerebral palsy. The definition of cerebral palsy has always precluded the inclusion of brain-based disorders that have an underlying potentially progressive etiology with respect to further pathologic changes [3]. Such CP mimics include both inborn errors of metabolism (IEMs) and neurodegenerative disorders that have a genetic, typically autosomal recessive or X-linked, etiology [38].

IEMs are a collection of rare genetic diseases that generally result from a deficiency of an intracellular component (eg an enzyme or transporter) of a metabolic pathway, resulting in the accumulation of a substrate or intermediate in a pathway and/or a reduced ability to synthesize essential compounds. A recent systematic review identified over 80 such disorders of which 67 (84%) were currently treatable. These disorders belonged to 13 different biochemical pathways and were individually rare in occurrence. Of note, 38 of the 67 (57%) treatable disorders could be identified on standardized first-tier screening tests that were collectively low cost (ie < $1000) in obtaining and involving easily accessible biological fluids (ie blood, urine, CSF) [38].

A plethora of progressive neurodegenerative disorders starting in infancy or early childhood have been identified. The genetic basis for many has been identified enabling precise diagnostic testing. An ever-changing array of targeted gene panels have both lowered the cost of testing and facilitated the rapidity of accurate diagnosis.

The initial misdiagnosis of a CP mimic is understandable on initial presentation. Progression, by definition, requires two-time points of observation. The individual rarity of these disorders precludes ready identification by the practitioner of an obvious suggestive clinical phenotype. Some characteristic diagnostic features (eg telangiectasias in ataxia-telangiectasia) only present later in the disease's natural course [39]. However certain features may raise the clinician's suspicion of the possibility of a CP mimic at the initial visit. These include the following; parental consanguinity, a previously affected family member, episodic worsening of symptoms, ataxic-hypotonic or dyskinetic cerebral palsy subtype, a lack of well-recognized cerebral palsy risk factors (eg prematurity, perinatal adversity, hyperbilirubinemia), and normal imaging (which occurs in 15% of cases of cerebral palsy) [40].

The recognition of a CP mimic is of importance due to the potential treatability for many of these disorders and the potential for familial recurrence. Obviously, both of these attributes are potent modifiers of the burden of illness at both an individual and family level.

Conclusion

From the above clinical narratives, the tremendous diversity of cerebral palsy is readily apparent. The multiplicity of pathways for causation, presentation and evolution renders the correct management of this disorder a substantial challenge to clinicians and health professionals. The obverse side of this challenge is the opportunity that this diversity offers for multiple points of interventions that; either prevent the occurrence in the first place, modify its impact, or ameliorate outcomes through targeted interventions that are individualized to the child and family. While no 'quick fixes' to cerebral palsy can be foreseen, the opportunity does exist in almost every instance to make a substantive difference that improves wellbeing holistically from the varying perspectives of health, function, participation, or quality of life.

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Cerebral Palsy: Epidemiology

31

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Abbreviations

CP	Cerebral palsy
GMFCS	Gross Motor Function Classification System
CMV	Cytomegalovirus
HIE	Hypoxic ischaemic encephalopathy
CCCP	Case Control Study of Cerebral Palsy and Perinatal Death
WARDA	Western Australian Register of Developmental Anomalies
EUROCAT	European Surveillance of Congenital Anomalies network

Learning Objectives

1. Describe the prevalence of cerebral palsy in high income countries.
2. Identify the motor types of cerebral palsy.
3. Discuss the methods of classification used in children with cerebral palsy.
4. Discuss the concept of causal pathways to cerebral palsy, including the range of risk and possible causal factors for early brain injury.
5. Discuss the pathways to cerebral palsy that include congenital microcephaly, including genetic and environmental influences.

Highlights

- Cerebral palsy (CP) is a common, lifelong physical disability. As well as being a disorder of movement and posture, children with CP often have associated impairments of cognition, sensation, behaviour and epilepsy.
- The prevalence of CP fluctuates over time, however a recent decline in rates in high income countries has been reported.
- The epidemiology of CP is complex and heterogeneous. A variety of known and unknown genetic and environmental risk factors span the period from pre-conception to infancy, interacting to form pathways to early brain injury.
- Identifying specific causal pathways to CP enables exploration of opportunities to interrupt the pathway, thus preventing cases of CP.

Introduction

The epidemiology of cerebral palsy (CP) involves study of the distribution and determinants of CP in specific populations, with the aim of identifying strategies for prevention of CP or to improve outcomes for those with this lifelong condition [1]. This chapter will (i) describe the prevalence and clinical profile of CP across the world but with a particular focus on Australia, as reported in the most recent Australian CP Register Report (published November 2018, birth years 1995–2012), (ii) outline a selection of risk factors for CP, and (iii) use a detailed case study of one risk factor, congenital microcephaly, to demonstrate the complexity of CP aetiology, including genetic and environmental influences. Subsequent chapters will further address the genetic influences and basic science of the development of CP, and the methods of diagnosis, neuroradiology and neuropathological findings of, and interventions available for children with CP.

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Definitions

CP includes a heterogeneous group of motor disorders. Current definitions and descriptions of CP are the result of contributions from individuals from a range of clinical backgrounds over a considerable period of time [2–6]. The widely used definition of CP is a clinical description that aims to define this condition in a manner that is relevant to a wide range of disciplines and highlights the numerous co-morbidities common to CP. CP is defined as: '***A group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of CP are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy, and by secondary musculoskeletal problems***' [7, p. 9].

CP registers contain data from a defined set of variables relevant to CP surveillance, collected for all individuals with CP born and/or living within a defined geographical region. These data may be used to identify risk factors and causal pathways to CP; to monitor changes in rates, clinical characteristics and severity over time; and to explore the 'real-world' impact of changes in clinical practice [8, 9]. This chapter draws heavily on data from CP registers and surveillance programmes across the world [10].

Prevalence

High-Income Countries

Overall, the prevalence of CP is reported to be approximately 2.0/1000 live births [11–13]. In the majority of cases (≈94%), the brain injury responsible for a child's CP will have occurred during the prenatal/perinatal period (throughout pregnancy and during the first 28 completed days after birth). These children are described as having pre/perinatal CP. Rates of CP amongst this group have varied over time, with birth prevalence in Australia fluctuating between 1.4 and 3.1 cases per 1000 live births since the 1960s [14]. The fluctuations occur as a result of changes in the spectrum (or profile, or distribution) of CP aetiologies and the often associated changes in clinical practice. For example, as advancing medical care enabled survival of increasingly preterm births, these survivors formed a new cohort at increased risk of CP; however, as management of very preterm infants improved over time, the risk of CP in this group has decreased [15]. There have however recently been reports of long-term declining trends in birth prevalence of CP from Europe (1980–2003) [16], Norway (1999–2010) [17], Australia (1995–2009) [18], Victoria, Australia (1983–2009) [15] and Japan (1998–2007) [19].

The Australian Cerebral Palsy Register reports rates of CP in Australia from 1995 onwards. The most recent data showed a decline from 2.1 per 1000 live births (95% confidence interval [CI] 2.0–2.3) in the mid-1990s to 1.4 (95% CI 1.3–1.5) in 2010–2012, the most recent cohort available [11].

Low- and Middle-Income Countries

There is emerging evidence to suggest that the prevalence of CP is higher in low- and middle-income countries, however population-based prevalence estimates are scarce [20]. The clinical profiles of CP are likely to differ from those in high-income countries, at least in part reflecting region-specific risk factors [20–22]. For example, low- and middle-income countries experience increased incidence of kernicterus, perinatal asphyxia and infections in infancy, which are more readily preventable in high-income countries, and lower preterm survival rates [9, 20, 21]. The epidemiology of CP in the context of low- and middle-income countries will become better understood in the coming years. Registers have recently been established in regions of Jordan [23], Bangladesh [21], Sri Lanka [11], Vietnam [22] and China, many of which share a minimum data set with the Australian CP Register or Surveillance of Cerebral Palsy Europe, facilitating future data sharing and comparison. The Bangladesh population-based register recently reported a prevalence of 3.4 per 1000 live births (95% confidence interval [CI] 3.2–3.7) [24].

Motor Type and Topography

A disorder of movement and posture is the defining feature of CP. The motor types of CP include spasticity, dyskinesia (dystonia, athetosis, choreoathetosis), ataxia and hypotonia. While children are generally described by their predominant motor type, many children with CP will exhibit more than one, e.g. spasticity and dystonia [25]. Reliable description of motor type is known to be challenging. Tools such as the Cerebral Palsy Description Form [11] guide assessors to systematically indicate the presence of each motor type in each region of the body, then describe the predominance of motor types. This method is clinically useful and enables epidemiologists to re-classify based on clinical observation as appropriate.

The most common predominant subtype is spasticity, present in approximately 85% of children with CP [11]. Children with spasticity have increased tone (increased resistance to movement, which is velocity dependent) and pathological reflexes (increased reflexes, or pyramidal signs) [26]. The topographical distribution of spasticity indicates the

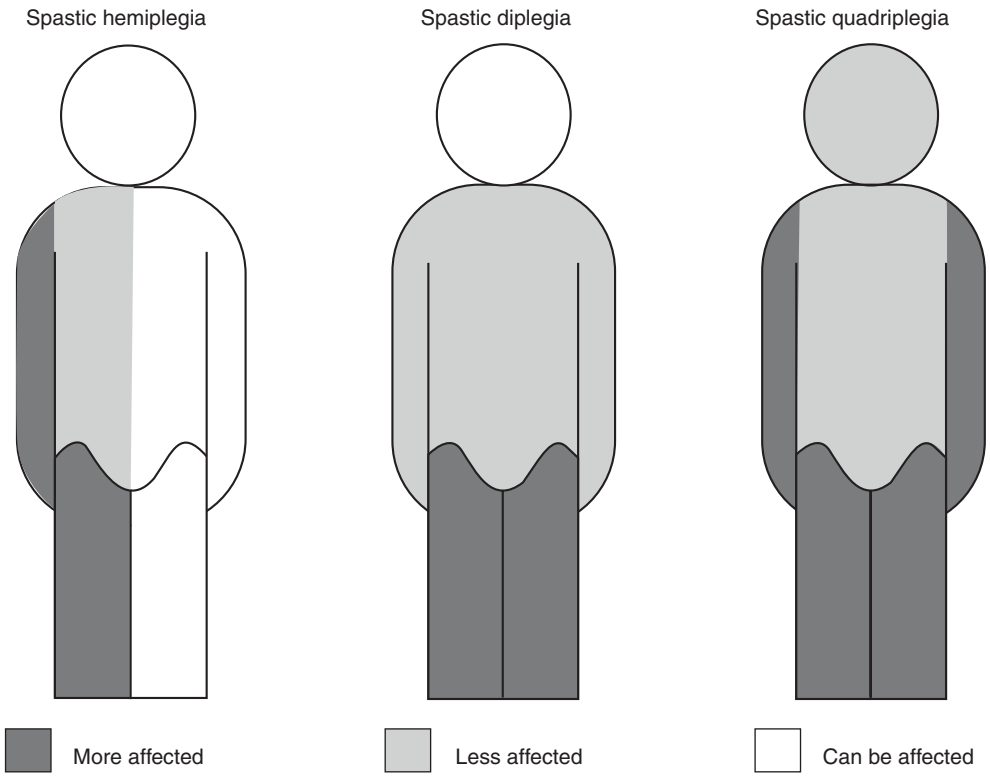

Fig. 31.1 Topographical pattern of spasticity in cerebral palsy (CP)

most affected region of the body (see Fig. 31.1): hemiplegia (or monoplegia) involves one side of the body, often with the upper limb more affected than the lower limb; diplegia affects both lower limbs, which are more affected than the upper limbs; quadriplegia (or triplegia) affects all four (three) limbs, with the upper limbs equally or more affected than the lower limbs and the trunk or orofacial muscles are also often affected. Unilateral spasticity (including hemiplegia and monoplegia) accounts for 40% and bilateral spasticity (including diplegia, triplegia and quadriplegia) for 60% of the spasticity subgroup [11]. Bilateral spastic CP is more common in children born preterm (<37 weeks; bilateral 69% vs unilateral 31%), while in children born at term the distribution is more even (bilateral 52% vs unilateral 48%) [11].

Dyskinesia includes, in isolation or combination: (i) dystonia (involuntary muscle contractions that may be sustained or intermittent muscle contractions causing twisting or repetitive movements and/or abnormal postures); (ii) athetosis (slow, continuous, involuntary writhing movements preventing maintenance of stable posture); or (iii) choreoathetosis (including ongoing, apparently random sequences of one or more discrete involuntary movements or movement fragments) [27]. Predominant dyskinesia accounts for approximately 7% of all CP cases, with recent estimates suggesting that at least 40% of children with dyskinesia will also have accompanying spasticity [11]. Predominant ataxia (accounting for 4% of all CP cases) is common in children with cerebellar pathology, while predominant hypotonia (3% of all CP) is the least common motor type, and is not recognised by some registers [11]. The distribution of motor type and topography also changes over time, in line with aetiological changes, with recent decreases in bilateral and dyskinetic subtypes in children with pre/perinatal CP [15, 28].

Classification Systems and Severity

Given the heterogeneity of CP, both in aetiology and clinical manifestation, meaningful and robust classification systems are vital to ensure consistency in reporting. These CP-specific classifications allow improved: description of the impairments and severity, prediction of current and future healthcare needs, comparison between different cohorts and evaluation of change at the individual and population level over time [7, 29]. In the last two decades, dedicated CP classification systems that are simple, valid and reliable have been developed. The Gross Motor Function Classification System (GMFCS) [30] was the first classification, and it is now in common usage throughout the world in clinical and research settings. Most children with CP (62%) are able to walk independently (GMFCS I–II), some require mobility aids to walk (11%, GMFCS III) and some use wheeled mobility (wheelchair) (26%, GMFCS IV–V). The proportion and rates of children with more severe gross motor function

Table 31.1 Presence of non-motor impairments in children with cerebral palsy (CP) [11]

Impairment	Some (%)	Severe (%)	Total: Any impairment (%)
Vision	29.4	4.4	33.7
Hearing	8.8	2.4	11.3
Speech	38.5	24.3	62.7
Intellect	26.1	19.5	45.6
Epilepsy	–	–	28.8
Epilepsy resolved by age 5	–	–	2.9

limitations (GMFCS III–V) has been decreasing in recent years in Australia [11, 15].

As implied by the definition, impairments of sensation, cognition, behaviour and epilepsy are common in children with CP [7], with recent Australian prevalence data reportedv in Table 31.1. Population-based studies have reported pain in 37% of children with CP [31], and 74% of adolescents with CP [32]. Pain is associated with more severe limitations of gross motor function [31, 33].

CP-specific classifications are now available for fine motor function (Manual Ability Classification System [MACS] [34] and Bimanual Fine Motor Function Measure [BFMF] [35]), communication (Communication Function Classification System [CFCS] [36] and Viking Speech Scale [VSS] [37]), feeding (Eating and Drinking Classification System [EDACS] [38]) and neuroimaging (Magnetic Resonance Imaging Classification System [MRICS] [39].

Risk Factors for CP

The vast majority (94%) of children with CP experience their brain injury during the pre/perinatal period [40], but rather than one isolated event, it is often the result of a sequence of interdependent events or conditions that eventually culminate in the brain insult underlying the CP [41]. These causal pathways to CP are complex and often poorly understood. Many risk factors have now been identified, spanning the period from pre-conception to the perinatal period. A selection of risk factors will be described.

Pre-Conception and Early Pregnancy

Risk factors existing prior to conception and in early pregnancy include maternal diagnoses such as seizures, intellectual disability and thyroid disease, inherited and de novo genetic mutations, young maternal age, low socio-economic status, primiparity, race/indigenous status, use of assisted reproductive technology, congenital anomalies and multiple birth [42–48].

Genetics

The understanding of genetic influence on risk of CP is a rapidly expanding area [43]. An indication of a genetic influence on at least some causal pathways to CP has been established, particularly as the relative risk of CP is modestly increased for siblings of a child with CP [49]. As males are consistently reported to have higher risk of CP, constituting 57% of all children with CP [11], this is further evidence of a genetic contribution to risk. This topic will be explored in detail in Chap. 35.

Maternal Age

The risk for CP is now highest in children born to teenage mothers [11], and the children with CP born to teenage mothers have more severe functional outcomes [50].

Assisted Reproductive Technology

Infants conceived with assisted reproductive technology, including in vitro fertilisation and intracytoplasmic sperm injection, have a two-fold increase in risk of CP, mostly mediated by the concurrent increase in preterm birth and multiple birth [46, 51]. With the change in practice towards single embryo transfer in some regions of the world, the risk of CP may be reduced by reducing the frequency of subsequent multiple and preterm births [52]. This may, however, be tempered by the overall increased use of assisted reproductive technology [53], and a small increased risk of monozygotic multiples after single embryo transfer [54].

Congenital Anomalies

The presence of a major congenital anomaly is one of the strongest risk factors for CP in children born at or near term [47, 55]. Anomalies range from minor to lethal, and may occur in isolation (one anomaly, or anomalies within one organ system), or across multiple body systems, sometimes in a recognised pattern constituting a syndrome, sequence or association. Higher proportions of both cerebral anomalies (e.g. lissencephaly, holoprosencephaly, microcephaly, hydrocephaly) and non-cerebral anomalies (e.g. congenital heart defects, hypospadias) have been reported [56].

Multiple Birth

Multiples have an increased risk of CP compared with singletons, with 12% of children with CP being a multiple com-

pared with the livebirth proportion of 3% [11, 57]. Risk has, however, decreased from the mid-1990s (8.2 per 1000 multiple neonatal survivors, compared with 1.9 for singletons) to 2010–2012 (4.7/1000 multiples vs 1.2 singletons) [11]. Higher-order multiples (triplets and beyond) are thought to have an excess risk compared with twins, but literature is limited since such births are rare [58, 59]. Infants who survived in utero death of a co-twin are also at increased risk of CP [60].

Pregnancy

Many complications during pregnancy are risk factors for CP, including haemorrhage, poly/oligohydramnios, preeclampsia, intrauterine growth restriction, intrauterine infection, maternal infection and placental abnormalities [41, 61, 62].

Congenital Infections

Congenital infections are frequently overlooked causes of neurodevelopmental disability. In recent years with outbreaks of Zika virus in South America and French Polynesia and the associated increased rates of congenital anomalies including microcephaly, the importance of intrauterine infections in neurodevelopmental disability has come into focus once more [63–65]. Infectious agents such as rubella, toxoplasma gondii and herpes viruses including cytomegalovirus (CMV) are well recognised as being capable of causing damage to the developing foetus and contributing to long-term neurodevelopmental disability including CP [66, 67].

Perinatal

Factors in the perinatal period that increase risk of CP include hypoxic ischaemic encephalopathy (HIE), sentinel events, stroke and low gestational age [61, 68, 69].

Hypoxic Ischaemic Encephalopathy

Hypoxic ischaemic encephalopathy (HIE) is reported in 21% of children with CP born at or near term [47]. In one Australian study, a potentially asphyxial birth event (sentinel event: most commonly tight nuchal cord, intrapartum haemorrhage or cord prolapse) was reported in 22% of children with CP following HIE, while inflammation (12%), growth restriction (15%) and congenital anomalies (25%) were also common in the same group. 'Birth asphyxia' now accounts for a much smaller proportion of CP than was historically believed [70], and the use of therapeutic hypothermia is further improving outcomes for children with HIE.

Low Gestational Age

The risk of CP increases with decreasing gestational age (Fig. 31.2). Despite this increased risk, the majority of children with CP are born at term, due to the relative rarity of preterm delivery in the population (~6–8%) [18].

Recent data from Australia indicate that the rates of CP are declining over time, particularly at term and at extremely low gestations. The rate of CP for children born extremely preterm (<28 weeks) has recently halved in Australia from 110.2 per 1000 neonatal survivors in 1995–1997, to 55.3 per 1000 neonatal survivors (2010–2012) [11]. The rate of CP for children born at term has recently fallen in Australia from 1.3 per 1000 neonatal survivors in 1995–1997, to 0.8 per 1000 neonatal survivors (2010–2012) [11].

These declines have led to a change in the gestational age group composition of the CP cohort. Previously (1995–1997), births at term contributed a greater majority of children with CP (60%), while the distribution is now (2010–2012) more even, with 53% being born at term, and a concomitant increase in late preterm (32–36 weeks) births (14–19% of cohort). The reduction in CP rates is likely multifactorial, underpinned by improvements in pre-conception, antenatal and neonatal care, including maternal magnesium sulphate prior to birth <30 weeks, antenatal steroids, improved resuscitation and caffeine for preterm infants. Other improvements include therapeutic hypothermia (with additional adjuvant therapies currently being tested), improved management of infants requiring surgery, single embryo transfer during assisted reproduction, and highly skilled and timely transfer of infants from remote locations to neonatal intensive care units [49].

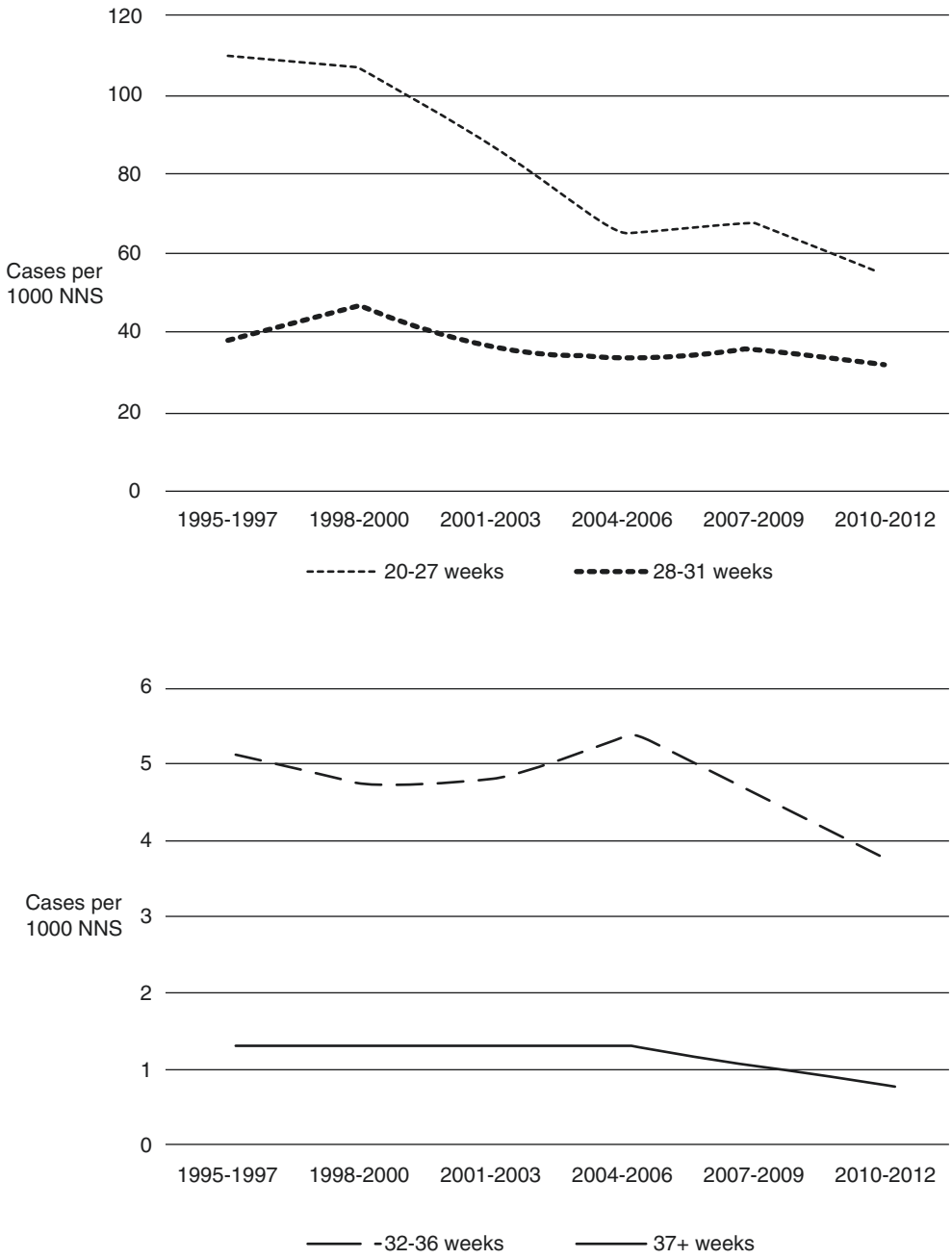

Fig. 31.2 Rate of pre/perinatal cerebral palsy (CP) per neonatal survivors (NNS), by gestational age in Australia (South Australia, Victoria and Western Australia combined) [11]

Post-Neonatally Acquired CP

For a smaller group of children (≈6%), their CP is due to a recognised post-neonatal injury acquired more than 28 days after birth and before two years of age. Amongst this group the rates of CP have also fluctuated over time, with recent declining trends reported in Europe (1976–1998) [71] and Victoria, Australia (1970–1999) [72].

The most recent data from Australia show that rates of post-neonatally acquired CP declined from 1.4 per 10,000 live births (95% CI 1.1–1.9) in 1995–1997 to 0.8 (95% CI 0.5–1.1) in 2010–2012 [11].

Common causes of post-neonatal CP include viral and bacterial infections (25%), cerebrovascular accidents (associated with surgery [10%], with cardiac complications [4%] or spontaneous/other [20%]), non-accidental injuries (12%) and post-seizure (5%) [11]. Children with post-neonatally acquired CP tend to have more severe associated impairments of vision, hearing, speech and intellect than children with pre/perinatally acquired CP [11].

In summary, CP is a common and complex neurodevelopmental condition. There are many recognised potentially causal factors that may interact to create many possibilities for early brain injury, with the possibility that many potentially causal factors still await recognition. A case study follows, to explore in more detail the impact of environmental and genetic influences on pathways to CP that include one important factor: congenital microcephaly.

An Examination of Microcephaly in CP: Including Genetic and Environmental Influences

As previously stated, congenital anomalies are an important risk factor for CP. The most common anomaly in children with CP is congenital microcephaly, reported in 2–3% of children in European CP registers [56, 73]. Congenital microcephaly refers to a smaller than expected head at birth. Given that head circumference is usually strongly associated with brain size, microcephaly infers a reduction in brain size [74]. On its own, however, it is not an accurate predictor of outcome, which can include normal development, intellectual impairment, epilepsy, ophthalmologic and audiologic disorders and CP [74–77]. Congenital microcephaly has multiple causes and causal pathways that can originate from genetic conditions including isolated gene defects and genetic syndromes, from environmental conditions, including prenatal infections, teratogens and exposure to maternal conditions or from gene–environment interactions [77, 78].

While congenital microcephaly is a common anomaly, objective definitions vary considerably. This may account for some of the variations in reported prevalence around the world: 1.5/10,000 in Europe [79], 5.5/10,000 in Western Australia [80] and 8.7/10,000 in the United States [81]. In children with CP, little has been reported about the pathways to CP that include congenital microcephaly. The rest of this chapter uses data from a total population case control study from Australia, with additional literature, to:

(i) Determine the prevalence of congenital microcephaly in an Australian population and percentage of children with CP who have congenital microcephaly, using a standardised definition that is reproducible in other populations
(ii) Determine the clinical outcomes of children with congenital microcephaly and CP
(iii) Hypothesise a number of potential genetic and environmental pathways to CP that include congenital microcephaly

Definition of Congenital Microcephaly

Congenital microcephaly is described when a newborn's occipito-frontal head circumference is smaller than expected, compared with gestational age and sex-matched normative data. However, inconsistencies exist within published literature and clinical practice. Firstly, the threshold used to define congenital microcephaly varies, with requirements of a head circumference: (i) more than three standard deviations (SD) below the mean for age and sex (≤3 SD) [79], sometimes described as 'severe' microcephaly; (ii) more than two standard deviations below the mean for age and sex (≤2 SD); [77] and (iii) less than the third or up to the tenth percentile for age and sex [81].

Secondly, there are over 100 published growth charts available for use, which affect the centile or *z*-score calculated from the infant's observed head circumference [82]. These include country-specific charts as well as charts based on a variety of populations, including the World Health Organisation's (WHO) Multicentre Growth Reference Study [83]. More recently, the INTERGROWTH-21st Project charts were published, which include optimal size at birth by gestational age in weeks and days, from 24 + 0 to 42 + 6 [82, 84].

Thirdly, variation also exists regarding additional formal and informal criteria required prior to allocating or reporting a description of congenital microcephaly. As population-based estimates of congenital microcephaly are often lower than the expected prevalence based on a Gaussian distribution of head size, it has been suggested that clinicians require evidence of pathology or clinical concern in addition to the small head circumference metric, prior to describing an infant as microcephalic [80]. The World Health Organisation's birth defects surveillance manual states that a head circumference less than two standard deviations for age and sex without evidence of structural abnormalities of the brain is not considered to be a major anomaly [85]. Further, as the aetiology of congenital microcephaly is genetic in a proportion of cases, some reports exclude microcephaly with a known genetic (including chromosomal) condition [79]. Some advocate for congenital microcephaly to be further dichotomised between proportionate, indicating similarity between centiles of head circumference, birth length and birth weight, and disproportionate, where head circumference alone (or with either birth length or birth weight) is smaller than expected [78]. Proponents of dichotomisation state that disproportionate microcephaly represents an indication of a pathologically small head, excluding infants who are simply non-pathologically small, e.g. due to small parental size. However, others consider that proportionate micro-

cephaly should not be excluded, as this could exclude infants with very early onset intrauterine growth restriction [74].

The Case Control Study of Cerebral Palsy and Perinatal Death (CCCP)

The CCCP includes all children with CP, a matched control and a representative sample of perinatal deaths born in Western Australia, 1980–1995. At the time of the study there were approximately 25,000 births per year in the state, with 75% of births in metropolitan hospitals [86]. The study was ethically approved by the Department of Health Western Australia and Princess Margaret Hospital (PMH) ethics committees.

Cases with CP were identified from Western Australian Register of Developmental Anomalies Cerebral Palsy (WARDA-CP: n = 740) and children with a known post-neonatal cause were not included. Controls (without CP), who survived the neonatal period, were identified from the Maternal and Child Health Research Database and were matched to CP cases for date of birth (within one year), gestational age at delivery (within one week) and plurality (n = 737). The perinatal deaths were not used for these analyses. Study data were extracted by trained, blinded data collectors from the child and mother's medical files held by obstetricians, general practitioners, hospitals of birth and hospitals of transfer. CP clinical outcomes were extracted from WARDA-CP, and data regarding co-occurring congenital anomalies (birth defects and congenital infections causing birth defects, coded by International Classification of Diseases, 9th revision with British Paediatric Association extension (ICD-9-BPA)) were obtained by linkage with WARDA-Birth Defects, a co-located, population-based register of birth defects identified by six years of age.

Anomalies reported by WARDA were re-classified manually to meet the definitions of major congenital anomalies of the European Surveillance of Congenital Anomalies network (EUROCAT) [87]. Each case with anomalies was then classified according to the EUROCAT hierarchical classification system of aetiology of congenital anomalies. The EUROCAT system was chosen because it is a published, long-standing and explicit classification system, which expands on broad aetiological classifications with consensus agreement [88].

For this study of congenital microcephaly in CP, it was important to ensure: that the growth standards were consistent throughout the study, that post-neonatally developing microcephaly was excluded (as this is common in children with CP) and that comparisons could be made for different thresholds of microcephaly. For these reasons, we allocated a description of congenital microcephaly based on head circumference at birth, as reported in medical files. The INTERGROWTH-21st International Newborn Size at Birth Standards application, which is recommended for use in Australia by the Australian Paediatric Surveillance Unit [89], was used. Head circumference, sex and gestational age at delivery were converted, using the application, into a head circumference z-score. Congenital microcephaly was assessed, based on thresholds of more than two, and more than three standard deviations below the mean for sex and gestational age.

Percentage and Prevalence of Congenital Microcephaly in Children with CP in the CCCP

When defined as head circumference ≤2 SD below the mean, congenital microcephaly was identified in 57 babies. This included 2.0% (n = 14) of controls, indicating that the INTERGROWTH-21st conversions led to our control population approximating the expected proportion of a normal distribution. By comparison, 43 (6.4%) children with CP had congenital microcephaly at birth (odds ratio [OR] 3.3; 95% CI 1.8–6.1) (Table 31.2). This study confirms that congenital microcephaly is at least three times more common in children with CP than in the general population.

When the stricter definition of ≤3 SD was used in the CCCP study, 0.4% (n = 3) of controls and 1.3% of CP (n = 9) cases were described as having (severe) congenital microcephaly. However, if congenital microcephaly is considered to refer to a pathologically small head, the more stringent criterion (≤3 SD) minimises the proportion of non-pathologically small heads (i.e. of false positives). In the current study since all subjects with CP must have some cerebral pathology, we are more concerned to include all those whose head size at birth is smaller than it should be (i.e. to minimise the proportion of false negatives). Therefore, the less stringent criterion of ≤2 SD is used in the remainder of this chapter.

Using this definition, all controls with congenital microcephaly were singletons, whereas 14% of the children with CP and microcephaly were twins, with one further microcephalic child with CP whose birth was registered as singleton because their co-twin died before the 20th gestational week (Table 31.2). The majority of all children with congenital microcephaly were born at term (≥37 weeks): 77% of CP cases and 64% of controls, and no children born extremely preterm (<28 weeks) were microcephalic. A smaller proportion of children with CP were disproportionately microcephalic (33%) than controls with microcephaly (50%), and it follows that more children with CP and microcephaly were clinically described as small for gestational

Table 31.2 Prevalence and description of children with congenital microcephaly

	Controls: *n* (%)	CP cases: *n* (%)	OR for CP cases compared with controls
Microcephaly ≤2 SD	**14 (2)**	**43 (6.4)**	OR 3.3 (95% CI 1.8–6.1)
Unknown head circumference (HC)	*47 (6.4)*	*73 (9.9)*	–
Head circumference proportionality	–	–	–
Disproportionate	7 (50)	14 (33)	OR 0.5 (95% CI 0.1–1.7)
Unknown	*0*	*1*	–
Gestational age (weeks)	–	–	–
Mean ± SD	*36.9 ± 3.9*	*38.0 ± 3.0*	–
Median	*38.5*	*39.0*	$U = 349$, $z = 0.905$, $p = 0.37$
24–27	0	0	$p = 0.23$
28–31	2 (14.3)	1 (2.3)	–
32–36	3 (21.4)	9 (20.9)	–
37+	9 (64.3)	33 (76.7)	–
Plurality	–	–	–
Singleton	14 (100)	37 (86.0)	1
Multiple	0	6 (14.0)	OR 5.0 (95% CI 0.3–95.1)
NICU/SCN admission 3+ days	–	–	–
Yes	5 (35.7)	13 (31.7)	OR 0.8 (95% CI 0.2–3.0)
Unknown	*0*	*2*	–
Clinically small for GA	–	–	–
Yes	2 (14.3)	14 (35.9)	OR 3.4 (0.7–17.2)
Unknown	*0*	*4*	–
Other congenital anomalies	–	–	–
Yes	0 (0.0)	19 (44.2)	OR 23.1 (1.3–411.7)

CI confidence interval, *CP* cerebral palsy, *GA* gestational age, *NICU* neonatal intensive care unit, *OR* odds ratio, *SCN* special care nursery, *SD* standard deviation

NB. Proportions do not include missing/unknown data

NB. 0.5 correction for all cells when calculating OR with a zero cell

age (36% vs 14%, though these differences were not statistically significant). No control with microcephaly had an additional congenital anomaly compared with 44% of children with microcephaly and CP. The combination of the presence of any congenital anomaly and growth restriction has been reported in previous findings from this study to be associated with the highest risk for CP in term and near term singletons [47].

Clinical Outcomes of Children with CP and Congenital Microcephaly

Comparing all children with CP, the proportion of term births was higher in those with congenital microcephaly (77%) than those without microcephaly (62%). The distribution of severity of CP and motor subtypes also differed between those with and without microcephaly (Table 31.3). Children with congenital microcephaly had more bilateral but less unilateral spasticity, and had more severe gross motor dysfunction (equivalent to GMFCS levels IV–V). Using an overall score for disability, incorporating cognition, sensory, motor impairments and epilepsy [90], those with congenital microcephaly also had more severe overall disability than children with CP without microcephaly.

Genetic and Environmental Pathways to CP That Include Congenital Microcephaly

Causal pathways to CP are often complex and multifactorial, with many risk factors involved. The purpose of this exploration is not to definitively attribute cause of CP to specific pathways via microcephaly, but to demonstrate possible interactions that include microcephaly and the clinical outcome of CP.

Table 31.3 Clinical outcomes of children with cerebral palsy (CP), with and without congenital microcephaly

	No congenital microcephaly (total, $N = 624$): *n* (%)	Congenital microcephaly (total, $N = 43$): *n* (%)
CP subtype		
Spastic unilateral	196 (31.4)	8 (18.6)
Spastic bilateral	302 (48.4)	29 (67.4)
Ataxic	49 (7.9)	1 (2.3)
Dyskinetic	71 (11.4)	5 (11.6)
Hypotonic	6 (1.0)	0 (0.0)
Motor severity		
Mild	225 (36.1)	13 (30.2)
Moderate	199 (31.9)	11 (25.6)
Severe	199 (31.9)	19 (44.2)
Unknown	*1*	
Disability score (1–12)		
Mild (1–4)	314 (50.4)	11 (25.6)
Moderate (5–8)	176 (28.3)	12 (27.9)
Severe (9–12)	133 (21.3)	20 (46.5)

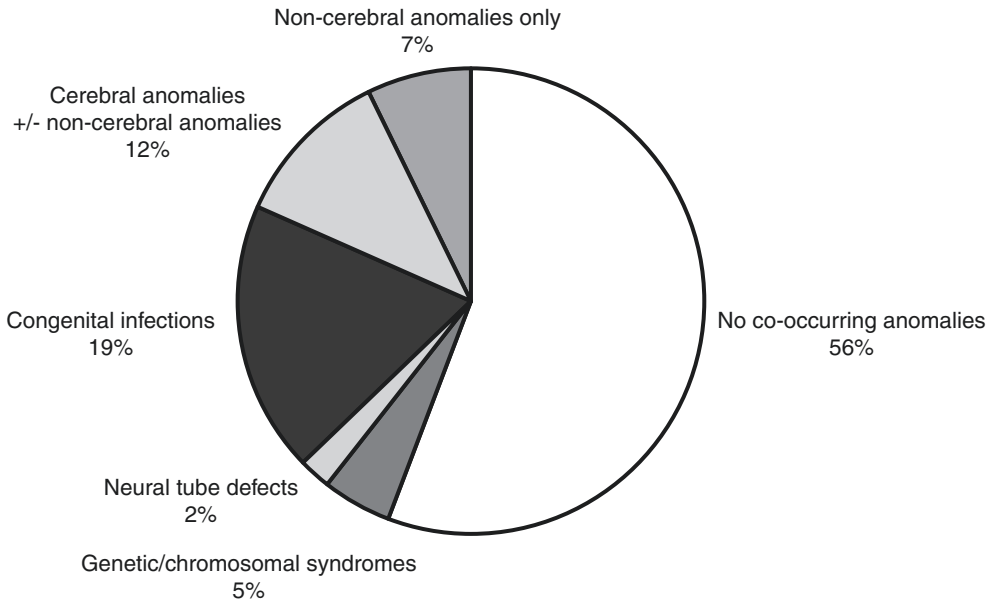

Fig. 31.3 Children with cerebral palsy (CP) and congenital microcephaly ($n = 43$): co-occurring congenital anomalies and infections

Genetic Pathways to CP Including Microcephaly: Known Genetic and Chromosomal Syndromes

Five percent of children with CP and microcephaly in the cohort, but no controls, were reported to have genetic or chromosomal syndromes. There is an ever-increasing understanding of the many genetic aetiologies of congenital microcephaly. Pathways can include chromosomal disorders and syndromes, contiguous gene deletion syndromes (e.g. 4p deletion Wolf-Hirschhorn syndrome, 22q11 deletion Velocardiofacial syndrome) and single gene defect syndromes (e.g. Cornelia de Lange syndrome, Smith-Lemli-Opitz syndrome) [77, 78]. As genetic testing is becoming more routine, it is probable that a greater proportion of cases with CP and microcephaly will be attributable to genetic or chromosomal syndromes.

Genetic Pathways to CP Including Microcephaly: With Additional Congenital Anomalies

Many cerebral anomalies, or maldevelopments, occur early in gestation [91]. In children with CP, these anomalies can be classified into disorders of cortical formation (proliferation and/or migration and/or organisation) and other maldevelopments (e.g. holoprosencephaly, Dandy-Walker formation, corpus callosum agenesis, cerebellar hypoplasia) [39]. The underlying causes of such maldevelopments are genetic and/or environmental (see below), and they may lead to congenital microcephaly at birth. In our cohort, 11.6% of children with CP and congenital microcephaly had co-occurring cerebral anomalies, including lissencephaly, pachygyria, holoprosencephaly and ventriculomegaly (excluding children with known teratogenic infections) (Fig. 31.3). An additional 7% of cases had co-occurring non-cerebral anomalies only (without known teratogenic cause). A proportion of these cases likely have an unknown genetic basis, which may contribute to the pathways to CP including microcephaly.

Environmental Pathways to CP Including Microcephaly: Congenital Infections

For 8 of the 43 (19%) children with microcephaly, CP and co-occurring anomalies, the anomalies could be attributed to a congenital infection (cytomegalovirus in 7 and rubella in 1). With the advent of immunisation programmes for rubella, congenital CMV is now the most common intrauterine viral infection in most developed countries [92]. The pathways to CP for these eight children are reported in Fig. 31.4.

The pathways to CP including congenital infections and congenital microcephaly ($n = 8$, Fig. 31.4) included singleton plurality for most children, while two children were co-twins. All but one child had other congenital anomalies, primarily cerebral anomalies, but only 25% of children had a clinical description of being small for gestational age. All children were born at term, and most received only routine care (75%) at birth. The motor outcome for the majority of children was bilateral spastic CP, with a severe overall disability.

CMV is a common infection. Most healthy people will not experience any symptoms, but a foetus is vulnerable should the mother experience an infection in pregnancy. Primary maternal infection holds the greatest risk for more severe foetal injury, particularly during the first trimester or early in the second trimester. Maternal infection and transmission of the virus to the developing foetus can result in congenital CMV disease and long-term disability: most commonly sensorineural deafness, but also epilepsy, intellectual impairment, visual problems and CP [93]. An estimated 10% of infants born with congenital CMV will be

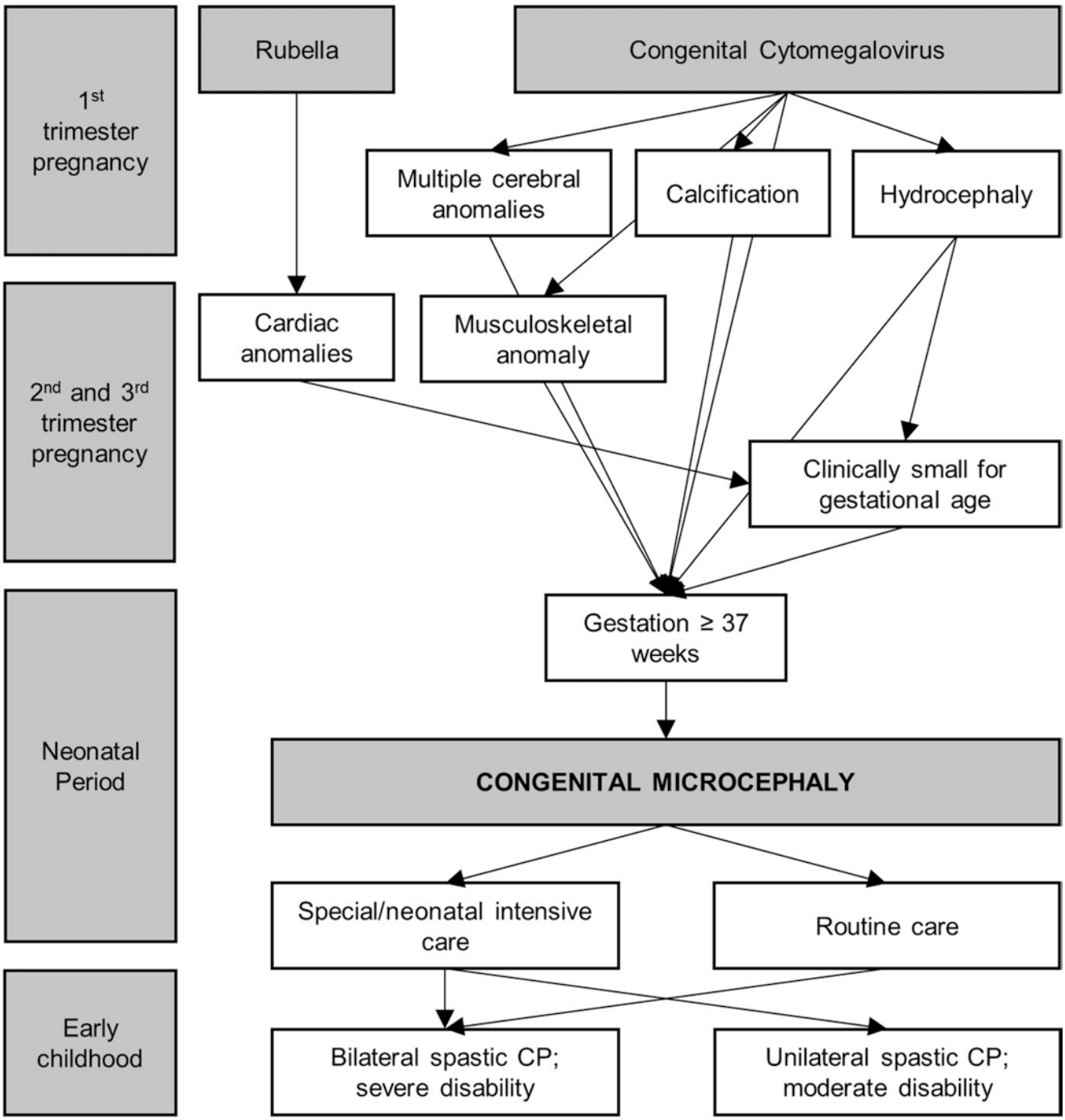

Fig. 31.4 Pathways to cerebral palsy (CP) in the Case Control Study of Cerebral Palsy and Perinatal Death (CCCP) study including congenital microcephaly and congenital infections ($n = 8$)

symptomatic in the neonatal period. Signs of neonatal symptomatic infection include microcephaly, jaundice, hepatomegaly, splenomegaly, petechiae, intracranial calcification, seizures, intrauterine growth retardation, prematurity, chorioretinitis and sensorineural deafness [93, 94]. As many of these signs are non-specific and most countries do not have routine screening for congenital CMV, some cases will be missed in the neonatal period when testing is most accurate. A recent study found neonatal CMV viremia in almost 10% of children with CP [95], compared with (<1%) in the general community [96]. Early identification of babies born with CMV disease is important to ensure monitoring of hearing over time, referral to appropriate early intervention services and, for some babies, antiviral therapy [97].

Prevention

The central tenet of the work to identify causal pathways to CP lies in the subsequent identification of prospects to interrupt these pathways. Opportunities to intervene at points along the pathway can be hypothesised and trialled, leading to primary and/or secondary prevention. Strategies that have already been proven effective and implemented widely include rubella vaccination, iodine supplementation in areas of severe iodine deficiency, cleaning environments contaminated with methylmercury and anti-D vaccination to prevent kernicterus [41]. Newer interventions, including magnesium sulphate for mothers at risk of preterm labour and therapeutic hypothermia with or without other adjuvant therapies such as erythropoietin for term infants with hypoxic ischaemic encephalopathy, are now being implemented in high-income countries.

There are also opportunities for prevention of some cases of congenital CMV. CMV is passed from person to person through contact with bodily fluids, so simple hygiene strategies can be adopted to reduce the risk of CMV infection during pregnancy (Fig. 31.5). These are particularly important for women in contact with young children, as the virus is common in children and can be shed in urine and saliva for up to two years after an initial infection [98]. As stated in the recent consensus guidelines, 'All pregnant women and healthcare providers should be educated about congenital CMV infection and preventive measures' [97, p. 2]. Unfortunately, awareness amongst pregnant women is poor and currently few (internationally less than 50% and in Australia less than 10%) maternity health professionals routinely discuss CMV prevention with women in their care [99–101]. Promotion of simple, clear information about CMV must be targeted to pregnant women and their families and support provided to health professionals to assist them in communicating these important prevention messages to women in their care. A link to a new video outlining these simple messages is provided: https://www.youtube.com/watch?v=Bh6WgbGvTd8.

Pathways to CP Including Microcephaly That Remain Unclear

The remaining 24 children (56%) with CP and microcephaly in this CCCP study had isolated microcephaly (Fig. 31.3), and the significance of congenital microcephaly on their pathway to CP could not be clarified. Other causes of congenital microcephaly reported in the literature include disruptive injuries (death of a monozygous twin, ischaemic and haemorrhagic stroke), teratogens (including alcohol, maternal phenylketonuria and poorly controlled maternal diabetes) and deprivation (maternal hypothyroidism, folate deficiency or malnutrition, and placental insufficiency) [77]. While we were unable to distinguish pathways to CP in our cohort of children with isolated microcephaly, we identified that the placenta of 45% of children with isolated microcephaly and CP (10 of 22 children; 2 unknown) was not healthy on macroscopic examination (vs 29% controls with isolated microcephaly: 4 of 14 children). Collecting retrospective data for pregnancy behaviours is notoriously difficult; however, in this cohort of children with isolated microcephaly, smoking during pregnancy was common (67% CP cases: 12 of 18, 6 unknown; 60% controls: 4 of 10, 4 unknown), while alcohol (21% CP cases: 3 of 14, 10 unknown; 13% controls, 1 of 8, 6 unknown) and recreational drugs (6% CP cases: 1 of 17, 7 unknown; 0 controls) were less frequent.

The Special Case of Postnatally Developing Microcephaly in CP

Postnatally developing microcephaly represents another condition, which has a different set of genetic (including inborn errors of metabolism and single gene defects) and environmental aetiologies [77]. Of particular interest in CP are pathways that include perinatal events such as hypoxic ischaemic encephalopathy, cerebrovascular accident and other brain injuries [77]. While the origins of this microcephaly may occur in utero or postnatally, the microcephaly is not present at birth and we therefore do not define it as congenital microcephaly in the context of CP. Postnatally developing microcephaly is common in children with CP, particularly in those with severe movement disorders, reportedly up to 68% of children [77]. Registers of congenital anomalies do not always differentiate between congenital and postnatally developing microcephaly, particularly if registration of anomalies occurs significantly after birth; indeed, studies of microcephaly often include postnatally developing microcephaly [74, 78]. In the context of CP and the elucidation of risk factors for this disorder, we advocate for differentiation of congenital (present at birth) and postnatally developing microcephaly in this population.

Fig. 31.5 Prevention strategy poster for cytomegalovirus infection during pregnancy. (Courtesy of Dr. Hayley Smithers-Sheedy)

Conclusion

Reducing the prevalence of CP will require improved care from pre-conception, through antenatal, perinatal and neonatal periods (particularly for pre/perinatally acquired CP), and care and public health messaging in early childhood (for post-neonatally acquired CP). The recent reduction in rates observed in Australia likely reflects the incremental impact of existing strategies. Ongoing surveillance through CP registers is vital to continue to investigate the changing epidemiology of CP over time.

Multiple Choice Questions and Answers

1. The prevalence of cerebral palsy (CP):
   a. Is similar in high and low-middle income countries, at approximately 2.0 per 1000 live births.
   b. Is similar in high and low-middle income countries, but fluctuates over time.
   c. Fluctuates over time, with recent increases in high-income countries due to the survival of extremely preterm infants.
   d. Fluctuates over time, with recent declines in high-income countries.
2. The four cerebral palsy motor types are:
   a. Spastic hemiplegia, spastic diplegia, spastic triplegia and spastic quadriplegia.
   b. Spastic hemiplegia, spastic diplegia, spastic quadriplegia and dystonia.
   c. Spasticity, dyskinesia (dystonia and choreoathetosis), ataxia and hypotonia, and children may have more than one motor type.
   d. Spasticity, dyskinesia (dystonia and choreoathetosis), ataxia and hypotonia, and occur in isolation in a child.
3. Severity classifications such as the Gross Motor Function Classification System, are useful as they can:
   a. Identify which children are at risk for microcephaly.
   b. Provide information regarding a child's CP motor type.
   c. Be used to simply describe the function of both individuals and cohorts over time and provide predictive information about future healthcare needs.
   d. Be used to describe all the clinical characteristics and associated impairments of children with CP.
4. Pathways to CP:
   a. Are clearly delineated, and always include both genetic and environmental factors.
   b. May include genetic and/or environmental factors, up to and including the time of birth only.
   c. Include either i) genetic or ii) environmental factors in isolation, which may exist from the period of pre-conception, through the pregnancy, and up to 2 years of age.
   d. May include genetic and/or environmental factors, which may exist from the period of pre-conception, through the pregnancy, and up to 2 years of age.
5. One pathway to CP includes congenital microcephaly:
   a. Which always co-occurs with another cerebral or non-cerebral congenital anomaly.
   b. Which is common in children with congenital cytomegalovirus, however the pathway may include other genetic and environmental factors.
   c. Which excludes children with congenital microcephaly and a known genetic syndrome.
   d. Which usually includes preterm birth and a disproportionate head size to birth weight and length.

Answers: 1(d), 2(c), 3(c), 4(d), 5(b)

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Diagnosis of Cerebral Palsy

32

Young-Min Kim and Stephen Ashwal

Learning Objectives

1. Understand how the concept of cerebral palsy (CP) has evolved over time and has various meanings today.
2. Recognize common "cerebral palsy syndromes."
3. Distinguish between the etiologic evaluation of CP and the individualized, needs-based evaluation of a person with CP.
4. Understand the value of an early diagnosis of CP.

Highlights

- Cerebral palsy is complex in its origins.
- Evaluating an individual child with cerebral palsy—requires an understanding of the etiology of CP and the individual's special needs.
- Evaluating individuals with CP occurs over the course of a child's development, from birth to adulthood.

Introduction

Cerebral palsy (CP) describes the spectrum of motor disorders secondary to a non-progressive abnormality in the developing brain. Akin to other neurodevelopmental disorders, the goal of diagnosing cerebral palsy is to relate a child's special needs to a body of knowledge that can be applied to optimize the child's participation in life activities.

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In this chapter, we highlight how the meaning of a diagnosis is its therapeutic value to the child and we highlight a framework for understanding the individual child with cerebral palsy.

Heterogeneity is intrinsic to cerebral palsy spectrum disorders. "Spectrum" does not describe a one-dimensional scale from mild to severe. The spectrum is multi-dimensional with regard to the etiology, phenomenology, distribution, co-existent conditions, and function. It is further elaborated by the child's unique personality, the characteristics of the family, the broader psychosocial and cultural context, and the spiritual beliefs through which disability is experienced. The spectrum is not only multi-dimensional but also dynamic—it changes with varying velocities across time as the child grows and develops. Thus each child possesses a developmental trajectory—a unique natural history and one's own narrative. The diagnosis of cerebral palsy ultimately signifies the opportunity to modify that trajectory and to engage in the moral enterprise of bettering a child's story.

Perspectives

Cerebral palsy is a common and accessible term that accommodates a wide range of perspectives. In its most basic and literal sense, it localizes the pathology to the brain (*cerebral*) and describes its manifestation of motor dysfunction (*palsy*). Historically, a seminal series of lectures titled *Cerebral Paralysis* in 1843 by William Little, an English orthopedic surgeon, attributed joint contractures and deformities to long-standing spasticity and paralysis of neurological origin and also hypothesized that preterm birth and perinatal asphyxia were the cause. Jakob Heine, a contemporary of Little and a German orthopedic surgeon, distinguished cerebral palsy from flaccid paralysis caused by poliomyelitis.

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Other early works by William Osler, Sir William Gowers, and Sigmund Freud also articulated that cerebral palsy was a motor disorder of cerebral origin [1, 2].

Winthrop Phelps in the mid-twentieth century pioneered the modern approach to caring for children with cerebral palsy and advocated for physical therapy, orthoses, and nerve blocks. He also advocated for a classification system based on mental and physical abilities as well as on an assessment of social function. He helped found the American Academy for Cerebral Palsy in 1947, which is now the American Academy for Cerebral Palsy and Developmental Medicine (AACPDM). A functional classification system, the Gross Motor Function Classification System (GMFCS), was developed in the 1990s by Rosenbaum and colleagues as an expansion of prior classification schemes that had focused mainly on etiology, typology, and distribution [3].

In the 2000s, high-quality neuroimaging advanced the practice of neuroanatomical correlation and the capacity for etiologic classification. Validated clinical instruments with predictive value such as the General Movements Assessment (GMA) and the Hammersmith Infant Neurological Examination (HINE) have led to the unprecedented ability to diagnose cerebral palsy in children less than five months of age with high sensitivity and specificity, leading to the recent initiatives for further research and implementation and of early diagnosis and early intervention for infants at risk [4, 5].

Persons with cerebral palsy are understood with different nuances in different contexts, and their care is commonly fractured and fragmented with no cohesive medical home. To neurologists, cerebral palsy may be primarily a matter of diagnosis—establishing the type of cerebral palsy, the localization of the lesion within the brain, and determining its etiology, either specifically or as simply non-progressive in nature. Orthopedic surgeons deal with the secondary musculoskeletal contractures and deformities. Neurosurgeons consider selective dorsal rhizotomy (SDR), intrathecal baclofen pumps, and deep brain stimulation (DBS). Physical, occupational, and speech therapists attend to an individual's functional-level and specific task-oriented goals. In school systems, a child's eligibility for special services is assessed. To the general public, persons with cerebral palsy may be mistakenly perceived as having intellectual disability [6–15].

Yet a person with cerebral palsy is not a sum of disparate medical problems but a human individual whose goals and abilities should be underscored continually. This highlights the imperative of understanding the person's and the family's perspective and helping them meet their goals. Enhancing the individual's participation in life activities—as described in the International Classification of Function, Disability, and Health for Children and Youth (ICF-CY)—is the ultimate purpose of our enterprise [16]. We recommend full utilization of the following definition and classification system in order to begin the journey of caring for a person with cerebral palsy.

Definition

Several attempts to formulate a universally accepted definition of cerebral palsy have occurred since the mid-twentieth century. These were led by groups such as "The Little Club" in the 1950s, the "Spastic Society" in the 1980s, and the "Surveillance of Cerebral Palsy in Europe" (SCPE) in 2000. In 1992, Mutch et al. described the following concepts that have endured in subsequent iterations: (1) Cerebral palsy is an umbrella term; (2) It is permanent but not unchanging; (3) It involves a disorder of movement and/or posture and of motor functions; (4) It is due to a non-progressive (i.e., "static") interference, lesion, or abnormality; and (5) The interference, lesion, or abnormality is in the immature brain [1, 17, 18].

> Cerebral palsy (CP) describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behavior, by epilepsy, and by secondary musculoskeletal problems.

In 2007, the above international consensus definition of cerebral palsy was published [19]. The definition includes two main concepts: cerebral palsy is first defined primarily as a non-progressive motor disorder localizing to the brain; cerebral palsy is then further elaborated as a syndrome that often co-exists with other neurodevelopmental disorders and medical conditions.

The consensus definition is inclusive of a "group" of conditions that are heterogeneous in etiology as well as the typology, distribution, and functional disability. It excludes conditions that are transient ("permanent") and non-disabling ("causing activity limitation") even when abnormal motor signs are present (such as asymmetric deep tendon reflexes with no functional consequence). It excludes developmental disabilities that do not impair motor function, i.e., it should not be conflated with intellectual disability, and we should abandon terms such as "mental retardation cerebral palsy" ("MRCP") that conflate the two.

Disturbances leading to cerebral palsy are presumed to occur before the affected function is expected to develop. As development occurs throughout childhood, limiting the time of the disturbance to the "fetal" or "infant" period is relatively arbitrary. Nonetheless, the first 2 to 3 years are generally considered more impactful, although there is no consensus upper age limit. Cerebral palsy is distinguished from brain

disorders that are progressive and cause an ongoing disturbance to motor function; thus cerebral palsy is due to a single, self-limited, or monophasic disturbance that is no longer active.

Cerebral palsy is a disorder of the *development* of movement and posture. This distinguishes cerebral palsy from similar motor disorders that are acquired after basic motor development is established. This developmental aspect highlights a key distinctive of pediatric neurology: in children, any lesion or disease process must be understood as applied to a dynamic and developing brain in contrast to the fully differentiated and developed adult brain.

The developmental nature of cerebral palsy highlights the importance of strategies aimed at improving trajectories rather than what is immediately apparent. Interventions can affect neuroplasticity and downstream consequences of cerebral palsy and thus reshape and redefine the condition. Cerebral palsy is dynamic—it evolves, being affected by the child's genetic predisposition, growth, development, learning, activities, psychosocial circumstances, medications, surgeries, overall health, and other personal and environmental factors.

Classic Cerebral Palsy "Syndromes"

Historically, cerebral palsy has been most commonly classified as clinical "syndromes" that describe the typology and the topographical distribution of affected limbs (e.g., spastic quadriplegia, spastic hemiplegia, spastic diplegia, monoplegia, paraplegia, double hemiplegia, triplegia, tetraplegia, generalized, etc.). Multiple classification schemes since Little's classification in 1862 have not successfully discriminated and defined cerebral palsy subtypes with universal agreement and application [1]. This is due to inherent ambiguities in the terminology, overlaps between subtypes, and implicit yet inconsistently present characteristics of the cerebral palsy "syndromes." The terms "diplegia" and "quadriplegia," for instance, have been used extensively to describe bilateral cerebral palsy, although these terms are imprecise and variably defined. Controversy remains on whether or not the terms should be abandoned altogether, and recent literature has adopted simply differentiating unilateral vs. bilateral involvement [20, 21].

Despite these controversies and the innate heterogeneity of cerebral palsy, several classic "syndromes" designated by the predominant motor disorder and limb distribution have been described and remain as fixtures in the clinical lexicon. They are generally useful in predicting functional (gross) motor impairment and predicting comorbidity burden and they also infer limited etiologic specificity and neuroanatomical correlation.

The interrater reliability between clinicians distinguishing both predominant motor disorder and limb distribution has been shown to be poor with less than 50% agreement [22]. Traditional terms describing bilateral cerebral palsy—"quadriplegia," "diplegia," "triplegia," "double hemiplegia"—are particularly confusing. For instance, for a person with bilateral cerebral palsy with left and right asymmetry as well as upper and lower limb asymmetry (e.g., as a result of asymmetric bilateral periventricular hemorrhagic infarction related to prematurity), one may designate "quadriplegia" based on involvement of all limbs, another may designate "triplegia" due to one limb being minimally involved or having no "appreciable" involvement, yet another may designate "diplegia" due to the predominance of lower extremity involvement (with some upper extremity involvement being "permitted"), and less commonly the term "double hemiplegia" may be employed to attribute the bilateral manifestations to separately localizing lesions. Epidemiological studies reflect this confusion, especially with the percentage of "diplegia" representing "spastic" cases ranging widely from 19 to 62% in European surveys involving comparably developed regions [21].

While traditional terminology has value in categorizing the broad range of cerebral palsy phenotypes and their pathophysiological correlations, we highlight the risk of overgeneralization and underappreciating their limitations. When it comes to the etiologic classification, these terms lead to a differential diagnosis, not the diagnosis itself. For instance, for "spastic diplegia," the leading causes are periventricular leukomalacia, intracranial hemorrhage, and intrapartum asphyxia, with approximately 40% of the cases having no identified cause [23]. Note also that "periventricular leukomalacia" and "intracranial hemorrhage" are themselves nonspecific and may result from different risk factors and mechanisms with variable distributions of injury and severity.

Spastic Hemiplegia/Unilateral Spastic Cerebral Palsy

Although lateralized hand-preference may be detectable as early as 6 months of age in normally developing infants, hand-preference normally stabilizes around 18 months [24]. Hemiplegia becomes obviously apparent by two years of age but can be appreciated earlier during acquisition of lateralized manual abilities in the early months when asymmetries should not be present (hold toy, hand to mouth around four months). In the first few postnatal months, asymmetries due to head preference and the tonic neck reflex should be discerned by examining patients with the head in mid-line position. Asymmetry may be appreciated by the quantity as

well as the quality of movements. Reaching across mid-line using the unaffected hand is a useful clinical sign.

Dysphasia related to left/dominant hemisphere disturbances, unilateral facial involvement, and homonymous hemianopia are uncommon in contrast to deficits due to later-acquired unilateral hemispheric injuries (which again highlights the developmental nature of cerebral palsy) [25]. Asymmetric cervical spinal cord lesions presenting (without cognitive, facial, and bulbar signs) may be falsely localized to the brain and designated as cerebral palsy.

Hemi-corticosensory impairments and hemineglect are common and are underappreciated [26]. Growth impairment of the abnormal side, related to decreased range and repertoire of motion, more often affect the distal parts of limbs. Limb-length discrepancy may interfere with gait mechanics and also lead to more complications involving the proximal leg, pelvis, and lower vertebrae. Nearly all patients with hemiplegic cerebral palsy have GMFCS levels I–III (i.e., ambulatory). Early intervention with constraint-induced movement therapy (CIMT) leads to better hand function than affected controls for infants with hemiplegic cerebral palsy, and this highlights the importance of early diagnosis in the modern era as well as the therapeutic value of designating unilateral vs. bilateral cerebral palsy [4, 27].

Spastic Diplegia/Bilateral Spastic Cerebral Palsy (Predominantly Affecting Lower Limbs)

Diplegic cerebral palsy implies predominant involvement of both lower limbs. Diplegia is the classic distribution of motor impairment related to prematurity due to the predilection toward white matter atrophy and periventricular white matter injury in the preterm brain (e.g., periventricular leukomalacia, germinal matrix hemorrhage, and venous infarction) [28, 29].

Neuroimaging is particularly important in diplegic cerebral palsy, particularly when upper extremity involvement and other impairments localizing to the brain are absent. Upper motor neuron syndromes affecting the lower limbs alone have a broader differential diagnosis including lesions localized to the spinal cord (such as tethered cord, spinal dysraphism, spinal tumors, transverse myelitis), and in the absence of brain abnormalities that correlate to the limb distribution, spinal imaging should be pursued. Many hereditary spastic paraplegias (HSP) and inherited dystonias present with lower extremity predominance [30].

Abnormal range and repertoire of movements in diplegic cerebral palsy lead to progressive contractures and deformities in the lower limbs as the child grows. Evidence-based hip surveillance guidelines (anteroposterior pelvic radiographs every 6–12 months starting at 12 months of age) allow early detection and prevention of hip dysplasia and hip displacement, and this highlights the therapeutic value of designating bilateral (either diplegic or quadriplegic) cerebral palsy vs. unilateral cerebral palsy [31, 32].

Common bone deformities affecting gait mechanics that may require orthopedic surgery include persistent femoral anteversion, external tibial torsion, and equinovarus or planovalgus deformities of the foot [33, 34]. When upper limb involvement is present (or "permitted"), asymmetry may be more pronounced in the upper limbs than the lower limbs. Motor disorders affecting upper limbs may be mixed and involve dyskinesias, dyspraxia, dystaxia, and decreased selective motor control. For children with GMFCS level III, ambulation may depend on the ability to use the upper limbs to hold assistive devices.

Spastic Quadriplegia/Bilateral Spastic Cerebral Palsy (Affecting All Limbs)

Quadriplegic cerebral palsy is characterized by generalized motor dysfunction affecting all limbs and often involving the trunk, neck, and bulbar function. Although spasticity is a form of hypertonia, persons with quadriplegia often have axial (truncal) hypotonia in the presence of appendicular hypertonia. In contrast, other individuals may present with generalized hypertonia.

The majority of persons with quadriplegia is classified in GMFCS level IV or V and experiences the higher incidence of co-existent medical conditions [35]. The diffuse nature of motor impairments generally correlates with the diffuse nature of overall impairments in spastic quadriplegia.

Dyskinetic (Extrapyramidal) Cerebral Palsy

Dyskinetic cerebral palsy is subdivided into choreoathetotic cerebral palsy and dystonic cerebral palsy depending on which hyperkinetic movement disorder predominates. Choreoathetosis and dystonia often co-exist. Dyskinetic cerebral palsy is classically the result of kernicterus, hypoglycemia, or acute hypoxic–ischemic brain injury in term or near-term newborns.

When the duration or severity of the hypoxic insult is relatively limited and leads to isolated basal ganglia and thalamic (BGT) injury (with sparing of the cortical gray matter), relatively pure dyskinetic cerebral palsy results. While the presence of basal ganglia and thalamic injury is highly predictive of "severe" (GMFCS IV and V) cerebral palsy and likewise severe communication impairment, BGT injury in isolation does not lead to cognitive impairment [36]. With use of augmentative or alternative communication (AAC)

and a supportive environment, these persons can possess a rich intellectual and social life.

In severe hypoxic–ischemic injury leading to diffuse cortical and subcortical injury (e.g., laminar necrosis pattern), a mixture of pyramidal and extrapyramidal signs occur. Dyskinetic cerebral palsy has the highest rate of normal neuroimaging, which may suggest a genetic cause when classic risk factors are absent [37].

Mixed Cerebral Palsy

Mixed cerebral palsy refers to the relatively equal predominance of both dyskinesia and spasticity. It conventionally does not refer to the distributive "mix" of axial hypotonia and appendicular hypertonia in quadriplegic cerebral palsy or the concomitant presence of ataxia.

Hypotonic Cerebral Palsy

Hypotonia is a non-specific finding and is poorly localizing in isolation; thus care should be taken in localizing hypotonia to the brain. Differentiating between central and peripheral (or combined) hypotonia is the first step in determining the etiology in conditions manifest with hypotonia, and a complete discussion with this regard is beyond the scope of this chapter. Over 500 genetic conditions are associated with hypotonia including trisomy 21, Prader-Willi and Angelman Syndromes, Joubert Syndrome, etc. [38]. Children with autism spectrum disorder often have low muscle tone. Hypotonia may be present in an otherwise normal child and may be confused with joint laxity. In general, a diagnosis of hypotonic cerebral palsy should alert the clinician to consider a genetic etiology, which may lead to an alternative diagnosis. Hypotonia may be transiently present as part of a developmental progression of infants with dyskinetic (as well as spastic) cerebral palsy as higher-level motor networks exert increasing influence.

Ataxic Cerebral Palsy

Like hypotonic cerebral palsy, ataxic cerebral palsy is relatively uncommon and should alert the clinician to consider genetic etiologies. Ataxic cerebral palsy is characterized by the predominance of cerebellar signs including nystagmus, dysarthria, dysmetria, truncal ataxia, and hypotonia. A cerebellar abnormality may or may not be demonstrable on neuroimaging.

Developmental Coordination Disorder (DCD)

A separate literature regarding developmental coordination disorder (DCD) describes approximately 5% of children with predominant motor difficulties that are described in less pathologic terms such as "clumsy" or "awkward." This diagnosis better suits persons with persistent delays in motor development and in persons with predominant motor dyspraxia (not accounted for by intellectual disability) as opposed to the motor disorders described above in relation to cerebral palsy. DCD is commonly co-existent with other neurodevelopmental disorders such as attention-deficit hyperactivity disorder, speech or language impairment, and learning disabilities [39]. In view of the evolving genetics of cerebral palsy and the shared risk factors between cerebral palsy and DCD (prematurity, small for gestational age, male sex), these two traditionally distinct disorders have increasing interface, and some argue that DCD and cerebral palsy are on the same continuum [40].

Diagnostic Evaluation

The diagnostic process in individuals with suspected cerebral palsy first entails determining the presence of a non-progressive brain lesion affecting motor function and its etiology. The process subsequently entails a thorough evaluation of the individual's goals, environmental factors, co-existent conditions, functional status, and all other factors related to the aim of providing therapeutic interventions. In other words, cerebral palsy, akin to intellectual disability and autism spectrum disorder, is a "gateway diagnosis." The initial cerebral palsy diagnosis leads to a more detailed evaluation to meet the specific needs of the individual person.

Etiologic Evaluation

Any brain disorder can be classified completely when the following four questions are answered: (1) Is the condition perinatal or acquired? (2) Is it progressive or non-progressive? (3) What is the underlying etiology? (4) What are the clinical manifestations? These questions must be addressed in order to have an adequate basis for caring for a child with cerebral palsy or any neurological disorder.

When assessing a child with cerebral palsy, a careful evaluation to determine whether the causative disturbance is static vs. progressive is paramount. When the perinatal (or early life) history, physical examination, and neuroimaging do not clearly establish an etiology, the natural history of the individual (with or without serial neuroimaging) may indi-

cate a static course. However, natural history alone has limited ability to indicate whether the underlying pathology is non-progressive as certain disorders are characterized by insidious progression or onset of progression at an older age.

The prognosis for all children with cerebral palsy is that they grow, develop, and learn (which is normative and applies to children universally). The trajectory is positive, albeit aberrant, and the outlook should be positive within realistic boundaries. Hence, cerebral palsy is a neurodevelopmental disorder in the sense that affected children continue to develop positively and that the meaning of the diagnosis is a consideration toward how to optimize that development. In contrast, a progressive condition indicates an ongoing disturbance that overwhelms the child's developmental potential leading to degeneration. This indicates a different prognosis and requires a different framework for setting goals and implementing interventions.

Cerebral palsy results from the interaction of multiple risk factors including genetic factors. In approximately half of individuals with cerebral palsy, perinatal risk factors cannot be identified [41]. In the preconception stage, risk factors for cerebral palsy include history of stillbirths, miscarriages, low socioeconomic status, African-American race, assisted reproduction, and abnormal genetic copy number variations. Risk factors are also detectable during pregnancy and include multiple gestations, male sex, intrauterine growth restriction, poly- or oligohydramnios, fetal anatomical anomalies, second or third trimester bleeding, intrauterine infections (i.e., (Toxoplasmosis, Other (syphilis, herpes varicella zoster, parvovirus B19), Rubella, Cytomegalovirus (CMV) and Herpes Simplex Virus) (TORCH) and human immunodeficiency virus (HIV) infections), preterm rupture of membranes, and maternal conditions including diabetes, alcohol and other substance abuse, epilepsy, thyroid disease, intellectual disability, and early or late maternal age [42, 43]. Perinatal risk factors include prematurity, breech and other non-vertex presentations, instrumental deliveries, pre-eclampsia, chorioamnionitis, meconium aspiration, placental abruption, uterine rupture, cord prolapse, and other causes of intrapartum hypoxia–ischemia. Postnatal risk factors include neonatal encephalopathy, sepsis, central nervous system or other systemic infections, hypoglycemia, acute anemia, hyperbilirubinemia, seizures, stroke, respiratory distress syndrome, postnatal corticosteroid use, and traumatic brain injury [44, 45].

When risk factors are identified, assumptions should not be made that they are necessarily causative. Likewise, in the absence of documented risk factors, a risk factor should not be presumed when there is no evidence (e.g., "the brain must have been deprived of oxygen at birth"). In instances where an adverse event (e.g., ischemic stroke involving motor tracts, postnatal meningitis, traumatic brain injury, etc.) clearly correlates with a significant disturbance that may lead to a motor disorder, the temporal relationship should be documented. Likewise, in the absence of clear causation (e.g., in the presence of normal neuroimaging), it should be documented that the cause is unknown, and appropriate diagnostic evaluations should be undertaken.

Both the history and physical examination are essential to localization and attributing a motor disorder to a brain abnormality. The presence of other brain disorders (e.g., intellectual disability, epilepsy, autism spectrum disorder) indicates a brain abnormality that is likely to be the origin of the motor disorder. An underlying etiology may be evident in the history, but the physical examination findings must correlate (e.g., dyskinetic cerebral palsy resulting from maternal placental abruption or severe neonatal hyperbilirubinemia, both of which cause basal ganglia injury).

One must consider both the typology of the motor disorder and its anatomical distribution to localize accurately. For instance, spasticity affecting both lower limbs can localize to the white matter in either the spinal cord or the periventricular brain regions. However, generally lesions localizing to the brain involve upper limb involvement to a lesser degree than the lower limbs. Dystonia, in contrast, always localizes to the brain. Hypotonia can localize virtually to any major region in the nervous system including muscle, nerve–muscle junction, nerve, spinal cord, and brain and it requires the preponderance of other brain-related deficits to be attributed to a brain abnormality. Ataxia is implicitly cerebellar in origin, but afferent ataxias related to sensory deficits also exist (e.g., Friedreich Ataxia).

Magnetic resonance imaging (MRI) shows abnormalities in 77–100% in selected (mostly retrospective) cohorts and is recommended in the neonatal period for those with established risk factors or at the time of presentation (level A) [46, 47]. Neuroimaging can confirm the brain localization and also the suspected etiology when classic findings (such as periventricular leukomalacia, porencephalic cyst, basal ganglia–thalamic injury) are found. Normal neuroimaging does not completely exclude cerebral palsy because factors such as timing since insult, myelination progression, extent and severity of abnormality, and the mechanism of disturbance can influence neuroimaging findings. The predictive value of term-equivalent brain MRI is discussed ahead in the "Early Diagnosis" section.

Computed tomography (CT) of the brain should be avoided in children, especially infants, due to the risk of malignancy related to ionizing radiation, except in special situations (e.g., evaluation for hydrocephalus or to determine the presence of calcifications). Cranial ultrasound may demonstrate cystic periventricular leukomalacia (PVL) in preterm infants with established risk factors for PVL but may not detect other lesions such as generalized white matter atrophy or cerebellar hemorrhage in the same individual.

When neuroimaging reveals developmental brain malformations, a genetic evaluation should be considered. In the presence of stroke, evaluation for coagulation disorders

should be considered (level B), but this requires a nuanced approach in the neonate as coagulation studies have limited value in the neonatal period. Otherwise, genetic and metabolic evaluations are not recommended in the routine evaluation of a person with cerebral palsy [46]. However, recent studies have revealed higher rates of de novo copy number variants (CNVs) in a population-based prospective cohort (7% compared to 1% in controls) and even a higher rate (31%) of clinically relevant CNVs in cerebral palsy of unknown cause [48–51]. Thus, when no etiology is determined by the initial evaluation (or if a family history of neurological disorders associated with cerebral palsy exists), genetic and metabolic testing should be considered (Fig. 32.1).

When cause and timing are not established, identifying conditions for which specific or disease-modifying treatments are available is of utmost priority (e.g., early onset islated dystonia (DYT5), treatable leukodystrophies). Other "cerebral palsy mimics" exist where the underlying disturbance is slowly progressive and/or portends a different prognosis (e.g., Hereditary Spastic Paraplegias, Spinocerebellar Ataxias). The diagnosis of cerebral palsy should be considered incomplete without an identified cause.

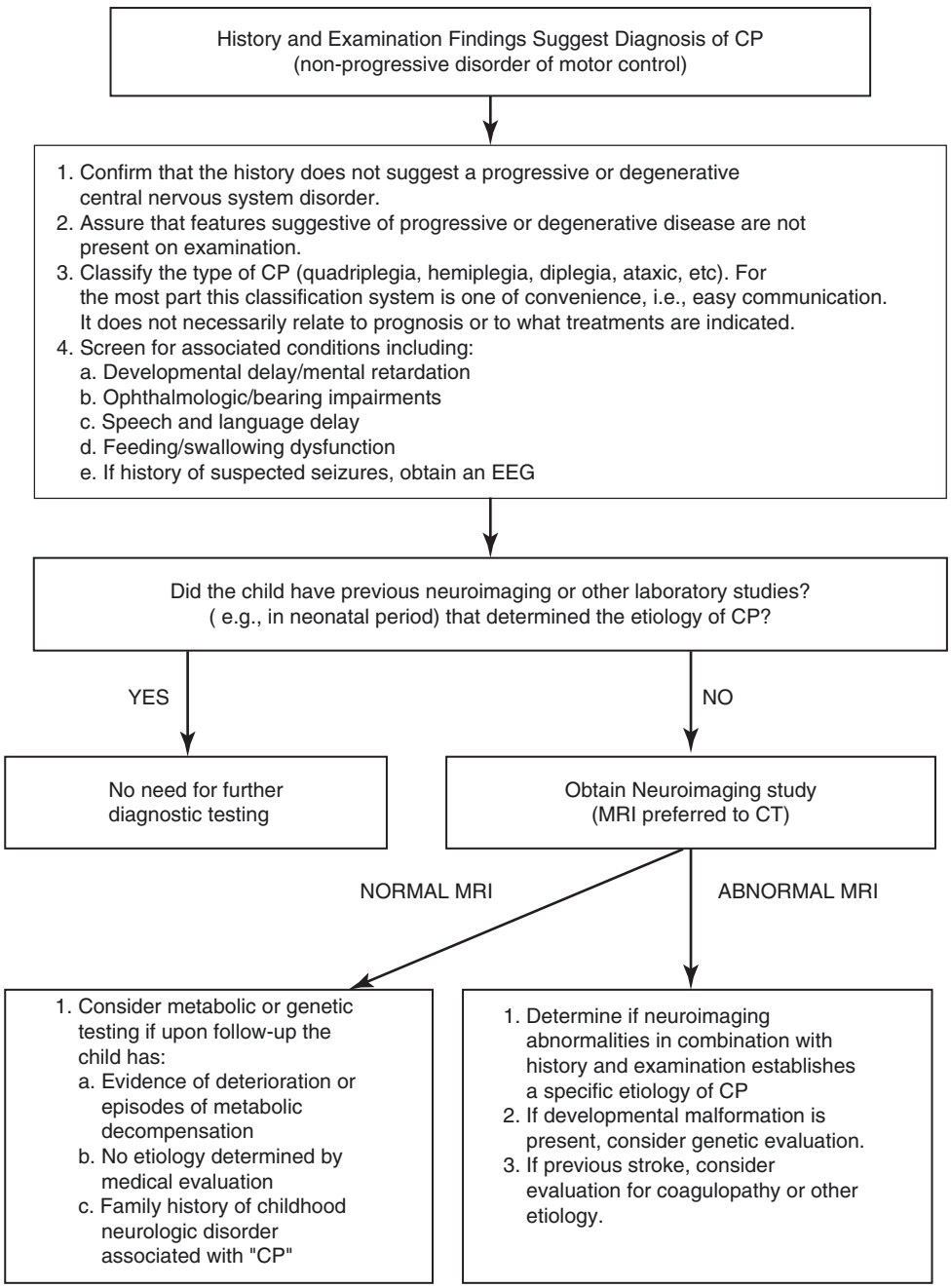


Fig. 32.1 Algorithm for the evaluation of the child with cerebral palsy. (Adapted from: Ashwal S, Russman BS, Blasco PA, Miller G, Sandler A, Shevell M, et al. Practice parameter: Diagnostic assessment of the child with cerebral palsy—Report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. *Neurology*, 2004;62(6):851–63)

Individualized Evaluation

In the care of the individual child, as each body region serves a particular set of functions, each limb, trunk, and the oropharynx should be described and treated individually in light of its motor abnormalities and its functional motor abilities and limitations in a multi-axial classification scheme. Furthermore, since motor functions leading to actual activities and participation generally are not isolated to a single body region (e.g., ambulating using a reverse walker involves not only the lower limbs but also the ability to grasp the walker stably using the upper limbs as well as stabilizing the trunk), each body region's contribution should be considered together in relation to each specific activity [52]. Treatments should be goal-directed so that the child can have enhanced performance and participation in life activities rather than directed primarily at a motor disorder, the degree of abnormal muscle tone present, or the mere appearance of a limb, or even specific clinical measurements.

The ICF-CY provides a model for describing function and disability in relation to a health condition as well as personal and environmental factors [16, 53] (Fig. 32.2). This framework can be applied to consider the relationships between body structures, their functions, activities, and ultimately the child's participation in life activities to guide interventions to help meet the child's goals [54–57].

Motor Abnormalities and Their Distribution

The *Reference and Training Manual* of the Surveillance of Cerebral Palsy in Europe (SCPE) divides motor abnormalities into three groups: spastic, ataxic, and dyskinetic; the dyskinetic group is further subdivided into dystonic and choreoathetotic groups [58]. In the care of the individual patient, characterizing motor abnormalities should be more detailed and include an assessment of negative motor signs (weakness, reduced selective motor control, ataxia, dyspraxia), an assessment of disorders of tone (hypotonia, spasticity, rigidity, hypertonic dystonia), an assessment of dyskinesias or hyperkinetic movement disorders (chorea, athetosis, choreoathetosis, hyperkinetic dystonia, tremor), and an assessment of contributions from musculoskeletal abnormalities [59–61]. In this light, essentially all persons with cerebral palsy have a "mixed" form where any or all of the above disorders affect motor function in qualitatively and quantitatively variable ways.

Negative motor signs may be overshadowed by other abnormalities in the mind of the examiner for several reasons. Because their treatments are predominantly activity-based, physicians may give more attention to disorders of tone that are more amenable to pharmacological or surgical treatment. When addressing an abnormal posture, one may fixate on a hypertonic muscle while neglecting the weakness of the opposing muscle (e.g., hypertonia and contracture of hip adductors or hip flexors in the presence of weakness of hip abductors or hip extensors, respectively). Because ataxia and dystonia can both have a "wobbly" appearance, the two may be mistaken for the other (although both can co-exist). Selective motor control is a fine motor correlate to weakness, which generally reflects gross motor function. The absence of negative motor signs portends a favorable prognosis because there is greater potential for active use and habilitation when disorders of tone and dyskinesias are treated or eliminated—the underlying abilities may be "unveiled" when the tone abnormalities are taken away (which may be particularly evident in SDR) [59].

Spasticity is a form of hypertonia, which is a disorder of tone. Tone is defined as a muscle's resistance to passive stretch, which is measured while the person is maintaining a

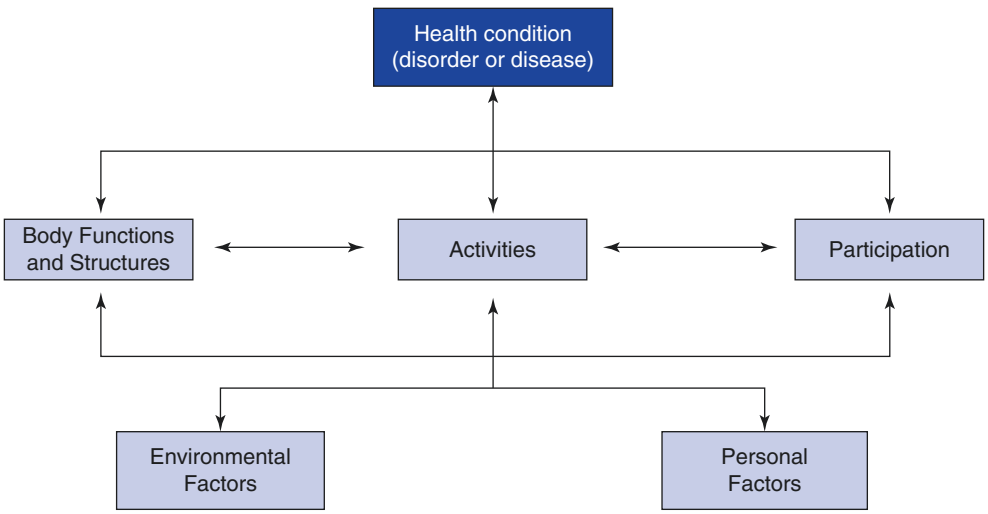


Fig. 32.2 A model of functioning and disability from the ICF-CY. (Adapted from page 17. http://apps.who.int/iris/bitstream/handle/10665/43737/9789241547321_eng.pdf;jsessionid=E3872BEF6408D2D0A1675EBFAA7267AE?sequence=1)

relaxed state of muscle activity. This definition excludes resistance attributable to musculoskeletal properties (such as fixed deformities and muscle and joint contractures). Spasticity is the abnormally increased resistance to passive stretch that is velocity- or angle-dependent [61]. Tone is assessed via manual examination where the resistance to passive stretch is felt by the examiner; spasticity, likewise, is assessed the same way using slow, medium, and fast velocities when stretching the muscle. Tone (and spasticity) cannot be assessed via visual inspection. Hence, while spasticity in the lower limbs may affect gait in characteristic ways, one cannot designate a "spastic gait" pattern via visual inspection alone. Gait patterns (e.g., equinus, equinovarus, scissoring, etc.) can have the same appearance when the primary disorder is spasticity or dystonia. Persons with dyskinetic cerebral palsy may have low tone when properly examined in a relaxed state.

Dyskinesias—conventionally referring to motor disorders of extrapyramidal systems and networks involving the basal ganglia, the cerebellum, and non-primary motor areas—are active (not passive) phenomena and are generally activated by action and posture [60]. They may represent a failure of the development of complex motor networks rather than a relatively simple disruption of the upper motor neuron leading to disinhibition of the spinally mediated deep tendon reflex. Dystonia is a commonly overlooked feature in children whose predominant motor disorder is spasticity—and the two often co-exist. When abnormal postures and resistance to passive range of motion are present inconsistently on examination, dystonia may be the cause (some may refer to dystonia as "dynamic tone," "dynamic contracture," or even falsely attribute it to behavior).

Both spasticity and dyskinesias are state-dependent and intensify in states of stress and diminish (or disappear) in states of rest or sleep (in contrast to fixed musculoskeletal contractures). While dystonia and spasticity often respond to the same treatment (e.g., botulinum toxin, baclofen, benzodiazepines), dystonia must be distinguished from spasticity with great therapeutic implications. Inherited dystonias may mimic the appearance of spastic diplegia by involving the lower limbs predominantly but they have drastically different etiologies and treatment (e.g., DYT5 responds exquisitely to levodopa supplementation). Spasticity may be permanently reduced via selective dorsal rhizotomy (SDR) whereas dyskinesias are not affected by SDR. Dystonia, particularly hypertonic dystonia, can be modulated by anticholinergic medications such as trihexyphenidyl [62]. Dyskinesias are far less prone to causing fixed musculoskeletal deformities and contractures as the range of motion is generally more preserved. Dyskinesias may be amenable to different early interventions to promote adaptive patterns of movement and motor learning. Deep brain stimulation (DBS) is an emerging treatment for dystonic cerebral palsy with modest benefits [63].

Musculoskeletal abnormalities pose deleterious effects on limb mechanics and can cause pain. Often tone abnormalities and contractures co-exist and must be distinguished as their treatments differ. The Modified Tardieu Scale (MTS) adheres to the definition of spasticity and measures the joint angle at the first reaction and the second, terminal resistance, which provides the functional range of motion (affected by neurogenic tone) as well as the passive range of motion (reflective of biomechanical properties, i.e., muscle length/contracture) [64, 65]. The Modified Ashworth Scale (MAS), in contrast, does not distinguish resistance due to tone vs. contracture and is highly subjective [64, 66]. Limitations in range due to fixed muscle contracture can improve modestly with stretching and pharmacological treatment (e.g., botulinum toxin injections) and may require serial casting or tendon-lengthening surgery to meet treatment goals.

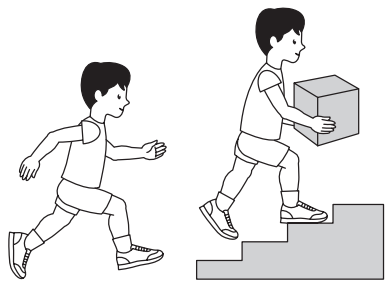
Persistent femoral anteversion, tibial torsion, and equinovarus or planovalgus deformities (among other deformities) are common in cerebral palsy affecting the lower limbs [33, 34]. These affect posture and limb mechanics and do not respond to treatments for spasticity and dystonia. Evidence-based hip surveillance guidelines should be implemented to prevent and to treat hip dysplasia and hip subluxation and dislocation, which is a time-sensitive matter because surgery becomes far more difficult once the triradiate cartilage closes. Infants with bilateral cerebral palsy who received regular surveillance and intervention have lower rates of hip displacement, contractures, and scoliosis [32].

Functional Classification

Functional classification provides a "picture" of the individual child with cerebral palsy and is also useful for risk stratification and surveillance for co-existing conditions. Functional domains to consider include gross motor (mobility, ambulation, transfer), fine motor (hand and arm function, bimanual abilities, self-care, non-verbal communication), and bulbar and oromotor function (swallowing, speech). Validated scales of function include the GMFCS, the Manual Ability Classification System (MACS), and the Communication Function Classification System (CFCS). These each designate ordinal values commensurate with functional ability on a non-overlapping scale from I (most able) to V (least able) (Fig. 32.3, Table 32.1). These systems provide insight with regard to disease severity, the quality and the quantity of a person's disability and service needs, and prognosis [67].

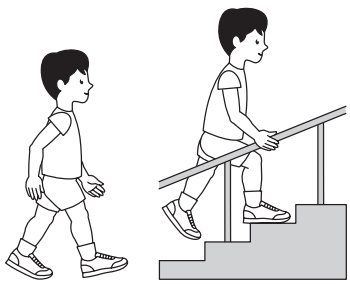
Communication ability should be distinguished from cognitive ability, just as other functional abilities (i.e., manual ability and gross motor ability) should be distinguished from other abilities [68, 69]. Communication may be verbal or non-verbal, and both depend on cognitive and voluntary

GMFCS E& R between 6 th and 12 th birthday: Descriptors and illustrations



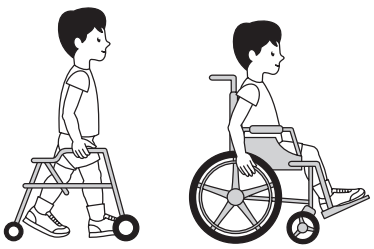
GMFCS Level I

Children walk at home, school, outdoors and in the community. They can climb stairs without the use of a railing. Children perform gross motor skills such as running and jumping, but speed, balance and coordination are limited.



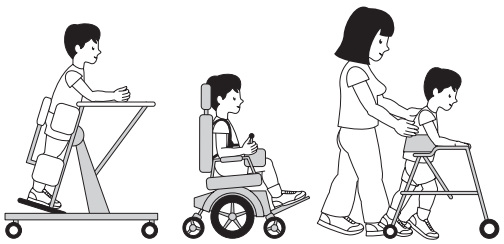
GMFCS Level II

Children walk in most settings and climb stairs holding onto a railing. They may experience difficulty walking long distances and balancing on uneven terrain, inclines, in crowded areas or confined spaces. Children may walk with physical assistance, a hand-held mobility device or used wheeled mobility over long distances. Children have only minimal ability to perform gross motor skills such as running and jumping.



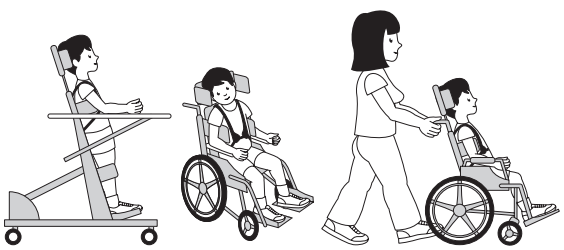
GMFCS Level III

Children walk using a hand-held mobility device in most indoor settings. They may climb stairs holding onto a railing with supervision or assistance. Children use wheeled mobility when traveling long distances and may self-propel for shorter distances.



GMFCS Level IV

Children use methods of mobility that require physical assistance or powered mobility in most settings. They may walk for short distances at home with physical assistance or use powered mobility or a body support walker when positioned. At school, outdoors and in the community children are transported in a manual wheelchair or use powered mobility.



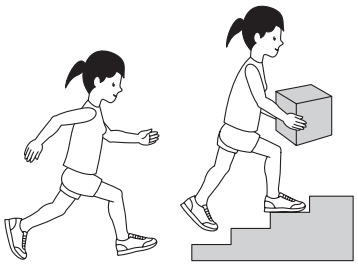
GMFCS Level V

Children are transported in a manual wheelchair in all settings. Children are limited in their ability to maintain antigravity head and trunk postures and control leg and arm movements.

GMFCS descriptors: Palisano et al. (1997) Dev Med Child Neurol 39:214–23
CanChild: www.canchild.ca

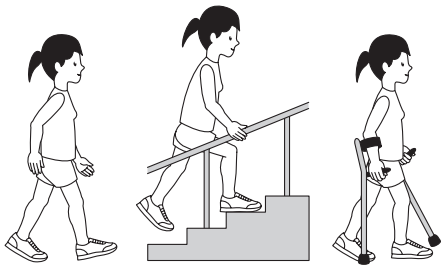
Fig. 32.3 GMFCS descriptors and illustrations. (Adapted from: https://canchild.ca/system/tenon/assets/attachments/000/002/114/original/GMFCS_English_Illustrations_V2.pdf)

GMFCS E&R between 12th and 18th birthday: Descriptors and illustrations



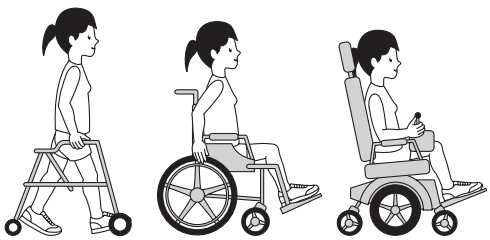
GMFCS Level I

Youth walk at home, school, outdoors and in the community. Youth are able to climb curbs and stairs without physical assistance or a railing. They perform gross motor skills such as running and jumping but speed, balance and coordination are limited.



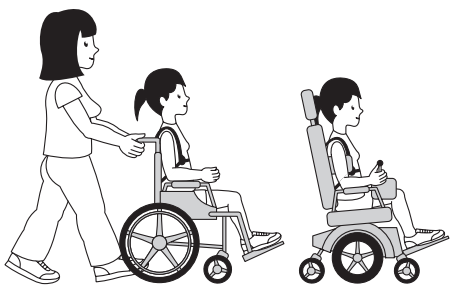
GMFCS Level II

Youth walk in most settings but environmental factors and personal choice influence mobility choices. At school or work they may require a hand held mobility device for safety and climb stairs holding onto a railing. Outdoors and in the community youth may use wheeled mobility when traveling long distances.



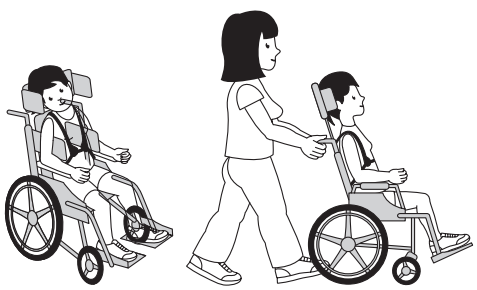
GMFCS Level III

Youth are capable of walking using a hand-held mobility device. Youth may climb stairs holding onto a railing with supervision or assistance. At school they may self-propel a manual wheelchair or use powered mobility. Outdoors and in the community youth are transported in a wheelchair or use powered mobility.



GMFCS Level IV

Youth use wheeled mobility in most settings. Physical assistance of 1–2 people is required for transfers. Indoors, youth may walk short distances with physical assistance, use wheeled mobility or a body support walker when positioned. They may operate a powered chair, otherwise are transported in a manual wheelchair.



GMFCS Level V

Youth are transported in a manual wheelchair in all settings. Youth are limited in their ability to maintain antigravity head and trunk postures and control leg and arm movements. Self-mobility is severely limited, even with the use of assistive technology.

GMFCS descriptors: Palisano et al. (1997) Dev Med Child Neurol 39:214–23
CanChild: www.canchild.ca

Illustrations Version 2 © Bill Reid, Kate Willoughby, Adrienne Harvey and Kerr Graham, The Royal Children's Hospital Melbourne ERC151050

Fig. 32.3 (continued)

Table 32.1 Functional levels of the Gross Motor Function Classification System (GMFCS), Manual Ability Classification System (MACS), and Communication Function Classification System (CFCS)

	Level I	Level II	Level III	Level IV	Level V
GMFCS	Can walk without limitations.	Walk with limitations	Walk with assistive mobility device	Walking ability severely limited even with assistive devices. Use of power wheelchair	Transported by manual wheelchair
MACS	Handles objects easily and successfully	Handles most objects but with somewhat reduced quality and/or speed of achievement	Handles objects with difficulty; needs help to prepare and/or modify activities	Handles a limited selection of easily managed objects in adapted situations	Does not handle objects and has severely limited ability to perform even simple actions
CFCS	Effective sender and receiver with unfamiliar and familiar partners	Effective but slower paced sender and/or receiver with unfamiliar and familiar partners	Effective sender and receiver with familiar partners	Sometimes effective sender and receiver with familiar partners	Seldom effective sender and receiver even with familiar partners.

Adapted from: Compagnone E, Maniglio J, Camposeo S, Vespino T, Losito L, De Rinaldis M, et al. Functional classifications for cerebral palsy: correlations between the gross motor function classification system (GMFCS), the manual ability classification system (MACS) and the communication function classification system (CFCS). *Research in Developmental Disabilities*, 2014;35(11):2651–7)

motor control. Individuals should be evaluated not only for verbal ability but also for their manual ability and oculomotor control because they may benefit immensely from alternative and augmentative communication (AAC). The CFCS considers all methods of communication for the affected person as both sender and receiver, including the pace and effectiveness of communication with both familiar and unfamiliar conversational partners. About half the persons with cerebral palsy have effective communication (levels I and II) and about one in seven are completely non-communicative (level V) [68].

Manual ability is affected in about three-fourths of persons with cerebral palsy, and about half of those affected have bilateral involvement. The distinction among levels I through V in the MACS depends on the quality and quantity of bimanual performance in daily living and the need for assistance or adaptation to perform daily tasks [70, 71]. About a third of persons with cerebral palsy have no manual limitations aside from that related to speed and accuracy (level I), and more than half of persons with cerebral palsy are in levels I–II. About one in seven persons with cerebral palsy have no functional manual ability [72].

The GMFCS has been extensively validated and proven to be psychometrically robust and stable over time [3, 73–75]. Levels I and II indicate the ability to ambulate without assistance. Level III generally indicates the need for assistance in order to ambulate (e.g., walker, crutches). Persons with GMFCS levels IV and V are wheelchair-bound. About half of persons with cerebral palsy are in level I, about 10% in level II, and about 12% in level III. The remaining one-third is equally distributed between levels IV and V. Up to 95% of persons with unilateral cerebral palsy or bilateral "diplegic" cerebral palsy are in levels I–III whereas only 25% of persons with bilateral "quadriplegic" cerebral palsy and dyskinetic cerebral palsy are in levels I–III [76].

Co-Existing Conditions

Persons who are non-ambulatory (GMFCS levels IV and V) or those with bilateral "quadriplegic" cerebral palsy or dyskinetic cerebral palsy are disproportionately affected by co-existing conditions including epilepsy, intellectual disability, hearing and vision impairments, communication impairment, feeding difficulties, respiratory insufficiency, risk of aspiration and pneumonia, gastrointestinal disorders, and musculoskeletal problems including scoliosis, hip subluxation, and pathological fracture [35]. These impairments may have direct effects on motor impairments (and vice versa), and may cause greater activity limitation than the motor impairment.

Non-motor impairments are generally common in cerebral palsy, either related to the same disturbance leading to the motor impairment or acquired secondarily as a developmental consequence [77–84]. A systematic review of co-occurring impairments, diseases, and functional limitations revealed that among children with cerebral palsy, ¾ were in pain, ½ had intellectual disability, 1/3 had severe intellectual disability, 1/3 could not walk, 1/3 had hip displacement, ¼ could not talk, ¼ had epilepsy, ¼ had behavior disorder, ¼ had bladder control problems, 1/5 had sleep disorder, 1/10 were blind, 1/15 were tube fed, 1/5 had excessive drooling, and 1/25 were deaf [79, 80] . A population-based study in Norway found that 57% of children with cerebral palsy had a psychiatric disorder, with attention-deficit hyperactivity disorder found in 50% of children [81–83]. Oculomotor dyspraxia (or "apraxia") is prevalent in about 55% of children with cerebral palsy [25, 85, 86]. Communication impairment was the strongest association of co-existent psychiatric conditions, but no association was found with intellectual disability or motor function. Addressing the individual's psychoeducational, hearing, vision, and communication needs; addressing problems related to pain, feeding, constipation, sleep, and musculoskeletal deformities; and addressing

epilepsy in those with paroxysmal events are essential and are recommended (level A) [46].

These compounded medical problems can lead to fragmentation of medical care and also places a great burden on caregivers in coordinating care. There also is increased risk of iatrogenic harm related to poly-pharmacy, treatments that do not meet the goals of the person affected, and conflicting recommendations from different specialists. This often overwhelming multitude of problems should be organized and prioritized, and management of these interrelated and complex co-existing conditions requires a coordinated interdisciplinary approach. Affected individuals also benefit from providers with expertise in caring for children with medical complexity (CMC).

Early Diagnosis

While historically cerebral palsy has been conventionally diagnosed after age two or three when its manifestations are clearly apparent, we are now in the era when accurate diagnosis of cerebral palsy is possible as early as less than five months of age [4, 87]. The combined use of identifying early risk factors, neuroimaging, and validated standardized motor assessments allows for early and accurate diagnosis of cerebral palsy. This allows for early diagnosis and early intervention that aims to change the child's trajectory at an early age and to optimize neuroplasticity and function.

When a diagnosis of cerebral palsy is uncertain, an interim diagnosis of "high risk of cerebral palsy" should be used to engage the child in cerebral palsy-specific early interventions. According to Australian cerebral palsy registry data from 1993 to 2009, false-positive diagnoses occurred in less than 5% of individuals, and almost all instances resulted in an alternative neurodevelopmental disability, not a normal developmental outcome [88]. Hence the diagnosis was meaningful in leading to interventions aimed at meeting the child's special needs. Moreover, population data also indicate that delaying the diagnosis is harmful to parents and caregivers, as they experience higher rates of depression, anger, and dissatisfaction with the prolonged diagnostic odyssey [54]. Early diagnosis, in contrast, leads to acceptance, increased confidence in the medical team, and improved access to interventions, resources, and communities with shared experiences.

Clinical Risk Factors

Clinical risk factors are reviewed in Sect. 5.1, under "Etiologic Evaluation."

Motor Dysfunction in Infancy

Motor dysfunction in infancy is ascertained via applying standardized and validated clinical tools. The infant's quality of movements may be abnormal (e.g., absent fidgety generalized movements between 10 and 20 weeks of postconceptional age), or neurological abnormalities may be apparent (e.g., early hand asymmetry or fixed postures). The infant's motor activities may be substantially below those expected for chronological age. These may be reported by the caregivers or observed via clinical assessment.

While clinical assessments have relied on the traditional neurological examination assessing axial and appendicular tone, head lag, ability to grasp, ability to reach, ability to sit, etc., two standardized motor assessments have been shown to have the best predictive validity [89]. Before 5 months of age, the Prechtl Qualitative Assessment of General Movements (GMs) has 98% sensitivity and is 95–98% predictive of cerebral palsy when combined with neuroimaging [5, 90–94]. The Hammersmith Infant Neurological Examination (HINE) can be up to 96% predictive of cerebral palsy before 5 months of age and has a sensitivity of 90% before or after 5 months of age [95–97].

Neuroimaging

Neonatal MRI has 86–89% sensitivity and can be combined with the above mentioned standardized motor assessments. When the trajectory of abnormal GMs or HINE scores produces findings congruent with MRI findings, predictive validity is even stronger. Cerebral white matter injury such as cystic periventricular leukomalacia or periventricular hemorrhagic infarction (i.e., "grade IV intraventricular hemorrhage"), cortical and deep gray matter lesions (basal ganglia–thalamic, watershed injury, multi-cystic encephalomalacia, or stroke), and developmental brain malformations (e.g., schizencephaly, polymicrogyria, cortical dysplasia, lissencephaly) are the most predictive [98].

In term hypoxic–ischemic encephalopathy, the severity of BGT injury is highly predictive of motor outcomes. When BGT injury is severe, the predictive accuracy is 83–96% for GMFCS IV and V. Abnormal posterior limb of the internal capsule (PLIC) can predict the inability to walk independently at two years (sensitivity 0.92, specificity 0.77, positive predictive value 0.88, negative predictive value 0.85) [36]. These findings predict motor outcomes but do not predict other outcomes such as cognition. As myelination is incomplete in infancy, MRI may not clearly distinguish white matter abnormalities.

Severity, Type, and Topography Indicators

In children two years of age or older, the GMFCS is predictive and remains stable into adulthood. However, prior to two years, severity is difficult to predict, and up to half of children have their GMFCS reclassified when reassessed. Milder presentations of unilateral cerebral palsy may score within the normal range on standardized motor assessments while still displaying lateralized abnormalities. Infants with bilateral spastic cerebral palsy (both diplegic and quadriplegic) display cramped synchronized movements followed by absent fidgety movements in the early months. Dyskinetic cerebral palsy presents with a poor repertoire of GMs followed by absent fidgety movements with circular arm movements and finger spreading.

Early Intervention

As any diagnosis ultimately carries therapeutic significance, early diagnosis ultimately means early intervention. Brain development and refinement of motor systems continues postnatally, and interventions to promote active movement are essential to drive motor cortex activity and motor learning, as well as to prevent aberrant development of brain networks and the musculoskeletal system [99].

Conclusion

In this chapter we highlight the importance of understanding the intrinsic diversity and the dynamism of persons affected by cerebral palsy spectrum disorders. The diagnostic process is iterative, continually re-evaluating as the child grows, develops, and learns and as the world around the child changes with new challenges and new treatments. This process informs our therapeutic and moral enterprise, following a child's journey as perspectives and goals develop and evolve.

The cerebral palsy spectrum is part of the human spectrum, operating in light of the undeniable and unique humanity each child possesses. To diagnose a child is to know the child and to try to make sense of that child's world. It is appreciating the child's best qualities and understanding the greatest challenges faced, aiming always for the best possible trajectory.

Multiple Choice Questions:

1. Which of the following concepts is NOT included in the definition of cerebral palsy (CP) per the 2007 consensus definition?
   a. CP is a disorder of movement and posture
   b. **CP cannot be of a genetic cause**
   c. Disturbances leading to CP are non-progressive
   d. CP is often accompanied by other neurodevelopmental disorders
2. Which of the following disturbances is associated with dyskinetic CP?
   a. Neonatal arterial ischemic stroke
   b. **Term hypoxic-ischemic encephalopathy**
   c. Cerebellar hemorrhage
   d. Periventricular hemorrhagic infarction
3. About what percentage of persons with CP have normal intelligence?
   a. 20%
   b. **50%**
   c. 70%
   d. 90%
4. About what percentage of persons with CP have epilepsy?
   a. **25%**
   b. 50%
   c. 75%
   d. 90%

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Neuroradiology of Cerebral Palsy

33

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Learning Objectives

- Review the causes of CP
- Review the available modalities of neuroimaging
- Discuss the typical neuroimaging pattern of some perinatal causes of CP: neonatal encephalopathy, intraventricular hemorrhage and periventricular hemorrhagic infarction, periventricular white matter injury, stroke, cerebral venous sinus thrombosis, bacterial meningitis, herpes simplex virus, and kernicterus
- Discuss the typical neuroimaging pattern of some prenatal causes of CP: congenital brain malformations, congenital cytomegalovirus, and congenital toxoplasmosis
- Discuss typical neuroimaging pattern of some postnatal causes of CP: non-accidental head injuries

Highlights

- The pattern of injury associated with neonatal encephalopathy can be subdivided into three main categories: basal ganglia injury pattern, watershed pattern, and near-total injury pattern.
- The extent of injury depends on the severity and duration of the event leading to the asphyxia and the timing of the neuroimaging.
- Head Ultrasound (HUS) is a good neuroimaging technique for early identification of Periventricular Leukomalacia (PVL) or Periventricular Venous Infarction (PVI), both of which tend to occur in the premature infant.
- MRI scans are the best modality for determining underlying metabolic abnormalities, such as kernicterus, or congenital abnormalities of the brain.

Introduction

Cerebral palsy (CP) is defined as the permanent disorders of the development of movement and posture that cause activity limitations [1]. CP can be subdivided according to the distribution of motor deficits and tone abnormalities into spastic CP (spastic diplegia, hemiplegia, or quadriplegia), dyskinetic CP (dystonia, athetosis, or chorea), ataxic CP, or mixed CP [2]. CP is the most common cause of neurodevelopmental disability in childhood [3]. Usually, CP is associated with different comorbidities, including epilepsy, intellectual disabilities, feeding difficulties, visual and hearing impairments, communications difficulties, and musculoskeletal impairments [4].

Typically, CP is attributed to non-progressive disturbances that occurred in the developing fetal, neonatal, or infant brain [1]. The underlying various etiologies for CP can

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Table 33.1 Possible etiologies of CP

Prenatal causes	Congenital brain malformation: de novo or inherited or as a result of infections, radiation, medications, or toxins (e.g., anti-epileptic drugs, alcohol) [5]
	Intrauterine infections: e.g., congenital cytomegalovirus, congenital toxoplasmosis, human immunodeficiency virus, varicella zoster, lymphocytic choriomeningitis virus, and herpes simplex virus (HSV)
	Stroke
	Chromosomal abnormalities: e.g., Aase, Aicardi, Angelman, Apert, Beckwith-Wiedemann, Cornelia de Lange, Di George, Fraser, Goldenhar, Incontinentia Pigmenti, Joubert, Noonan, oro-facial-digital, Prader-Willi, Salomon, Smith-Lemli-Opitz, Stickler, and Sturge-Weber syndromes [6]
Perinatal causes (80%)	Neonatal encephalopathy
	Intraventricular hemorrhage (IVH) and periventricular hemorrhagic infarction (PVHI)
	Periventricular white matter injury (PWMI)
	Stroke
	Cerebral venous sinus thrombosis
	Central nervous system (CNS) infections: e.g., bacterial meningitis (Group B streptococcus [GBS], *Escherichia coli*, *Listeria monocytogenes*, *Enterobacter*), neonatal herpes simplex virus (HSV), chorioamnionitis
	Kernicterus
	Neonatal hypoglycemia
	Non-cerebral malformations: Congenital heart disease, diaphragmatic hernia, facial clefts, limb and skeletal malformations, malformation of the face and skull bone [6]
Postnatal causes	Accidental and non-accidental injury (abusive head trauma)
	CNS infections: e.g., bacterial meningitis (Group B streptococcus, *E. coli*, *L. monocytogenes*, *Streptococcus pneumoniae*), herpes simplex virus, sepsis
	Stroke
	Anoxic insults: Near drowning
	Status epilepticus sequelae

be categorized broadly into prenatal causes, perinatal causes, and postnatal causes (Table 33.1).

Perinatal causes, which are responsible for around 80% of the underlying etiologies for CP, include neonatal encephalopathy, central nervous system (CNS) infections, stroke, and kernicterus [7]. Other recognized risk factors for CP are prematurity, intrauterine growth restriction, and chorioamnionitis. Prematurity especially is a well-known cause for CP, and it can cause CP through different complications related to prematurity such as intraventricular hemorrhage (IVH), periventricular hemorrhagic infarction (PVHI), and periventricular white matter injury (PWMI) [8]. Prenatal causes can include congenital brain malformation, intrauterine infection, stroke, and chromosomal abnormalities [7]. Postnatal causes can include accidental and non-accidental trauma injuries, CNS infection, stroke, and anoxic insults [7].

CP is mostly a clinical diagnosis based on a child's history and physical examination. However, with the advent of advanced neuroimaging, different imaging modalities—including head ultrasound (HUS), computed tomography (CT), and magnetic resonance imaging (MRI)—are now used to evaluate children with CP.

Available Imaging Techniques

Head Ultrasound (HUS)

Compared to other more advanced neuroimaging techniques, HUS has several advantages of use in neonates and infants with open fontanels, which includes easy access, decreased cost, and no associated radiation; HUS can be performed conveniently at the bedside of neonates, it does not require sedation, and it can be repeated easily several times if needed. However, the quality of the images is not as precise as a brain MRI, and the fontanel window may also limit the observation of the complete extent of brain injury. HUS can help to identify different brain pathologies including intraventricular hemorrhage, calcifications, ischemia, and brain abscess [9].

Head Computed Tomography (CT)

The advantages of a head CT are easy access for sick neonates [10] and lower cost compared to MRI; it also is a quick exam, for which sedation mostly is not needed in the neonatal period. However, radiation is the main disadvantage and limiting factor of this neuroimaging modality with respect to the pediatric population, which explains why it is used less and less with the advancement of MRI techniques.

Brain Magnetic Resonance Imaging (MRI)

Brain MRI represents the most precise diagnostic tool in neurology in general, and also in neonatal neurology. Today, it is widely available in developed countries, and its availability is beginning to spread to low-income countries. Sequences initially developed for adults have been adjusted for neonatal and infant brains. The main limitation of this technique is that most neonatal and infant protocols remain quite lengthy, during which motion should be limited to ensure high-quality imaging. Thus, approaches have been developed to limit the use of sedation for neonates and infants undergoing an MRI exam [11]. The progress of MRI techniques has contributed enormously to our understanding of normal brain development and the underlying mechanisms of brain injury in the neonatal population. In normal

term neonates, structures such as the posterior limb of the internal capsule (PLIC) and the corticospinal tracts (CSTs) in the brainstem are myelinated at term-equivalent age (TEA) [12], and thus appear hyperintense on T1-weighted imaging and hypointense on T2-weighted imaging [12], which typically serve as landmarks to differentiate pathological findings from normal findings.

Typical Neuroimaging Pattern of Some Perinatal Causes of CP

Neonatal Encephalopathy

Neonatal encephalopathy is a condition that is associated with high mortality and morbidity including CP [13]. Three main patterns of brain injuries have been described when this condition affects near-term/term neonates, depending on the type and severity of the initial asphyxial event (Table 33.2) [14, 15].

The basal ganglia injury pattern is characterized by the involvement of the ventrolateral (VL) thalami, basal ganglia (i.e., caudate head, globus pallidus, and putamen), and perirolandic cortex [15]. This specific pattern typically results from a severe acute hypoxic–ischemic insult such as abruption placenta, uterine rupture, or cord prolapse [16], which leads to injury to the most metabolically active tissues. It is also described as central cortico-subcortical involvement [14, 15]. The watershed injury pattern is characterized by involvement of the territory between cerebral arteries (i.e., between anterior and middle cerebral arteries [MCAs] and/or between posterior and middle cerebral arteries) [15]. Typically, the watershed injury pattern appears secondary to a reduction in blood flow and/or oxygen for prolonged duration [16]—in the context of hypotensive episodes, infections and hypoglycemia—that leads to shunting the blood to the most metabolically active tissues (preservation of the basal ganglia) and injury to these most vulnerable border zones [15, 17]. Parieto-occipital and posterior temporal regions are affected more often than anterior regions [14]. The near-total injury pattern (Figs. 33.1 and 33.2) is characterized by the involvement of most of the white matter and cortex [14]; thalamus and basal ganglia also may be injured. Typically, this injury pattern results from prolonged and severe hypoxia [14, 16]. The involvement of the cerebellum also has been described in the context of neonatal encephalopathy [18].

Table 33.2 Patterns of hypoxic–ischemic injury according to the severity and duration of the initial event

Severity and duration of the initial event	Description of the event	Brain injury pattern
Mild to moderate		
Acute (<10 min)	Acute partial ischemia and/or hypoxia, common during childbirth, that usually does not lead to significant clinical or imaging sequelae	–
Prolonged (10–25 min)	Prolonged partial ischemia and/or hypoxia; adequate shunting maintains blood flow to most vital structures, which leads to injury in peripheral or watershed territories	Watershed injury pattern
Severe (profound)		
Acute (<10 min)	Acute profound ischemia and/or hypoxia; inadequate shunting, with most metabolically active regions being the most susceptible to injury	Basal ganglia injury pattern, including thalamus, basal ganglia, and perirolandic cortex
Prolonged (10–25 min)	Prolonged and catastrophic profound ischemia and/or hypoxia, despite sometimes not being clear in the infant's history	(Near-)total injury pattern, affecting (near-)total brain; also called sometimes "white cerebrum"

Adapted from Ghei SK et al. [14]

HUS has been used to evaluate brain injury in the context of neonatal encephalopathy, since it can provide clues to the extent of injury [19]. Typically, HUS might be negative in the first 24–48 h of the injury [20], and the injury may only become apparent after the first 24–48 h, depending on the severity of the injury [21]. An injury manifests on HUS as an increased echogenicity in the white matter, gray matter, and/or basal ganglia [22]. Other findings suggesting parenchymal edema include the effacement of cerebral sulci, compressed slit-like ventricles and narrowing of the interhemispheric fissure, and basal cisterns [9].

A head CT is the least sensitive technique for evaluating neonatal encephalopathy, because of its poor contrast resolution, which is a result of the high water content of the neonatal brain parenchyma vs. the high protein content of the cerebrospinal fluid. The variable CT findings of hypoxic–ischemic injury can range from a subtle generalized loss of gray–white matter differentiation to a bilateral, symmetrical, low-density generalized cerebral edema [23]. A "reversal sign" may be seen with a higher attenuation of white matter compared to edematous gray matter [24]. A "white cerebellum sign" may be seen due to the abnormally hypodense appearing cerebrum secondary to edema that contrasts with a normal density cerebellum [25]. These last two signs are associated with a severe and poor neurological outcome [26].

A brain MRI is the most sensitive and specific diagnostic modality available for assessing the brain injury associated with neonatal encephalopathy. In this context, typically reported abnormalities [15] include the loss of normal signal

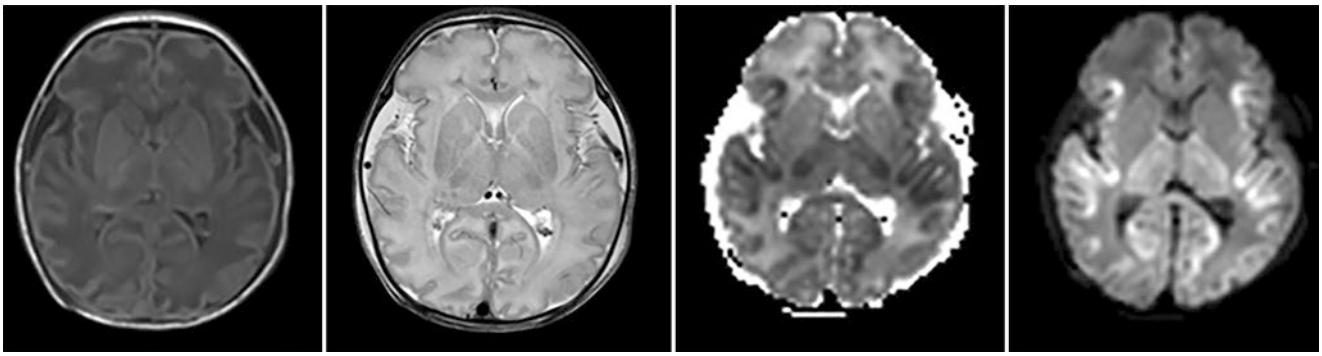

Fig. 33.1 Brain MRI (axial T1- and T2-weighted imaging, apparent diffusion coefficient [ADC] map, and diffusion-weighted imaging) performed on day 2 of life in a term neonate with neonatal encephalopathy. There is bilateral symmetrical diffusion restriction along the thalami and extensive bilateral cortical diffusion restriction involving almost all the lobes. There is no corresponding signal intensity change on T1- and T2-weighted imaging, except loss of the normal T1-hyperintensity along the posterior limbs of the internal capsules (PLICs), with corresponding diffusion restriction. The abovementioned imaging features are characteristics of severe near-total injury

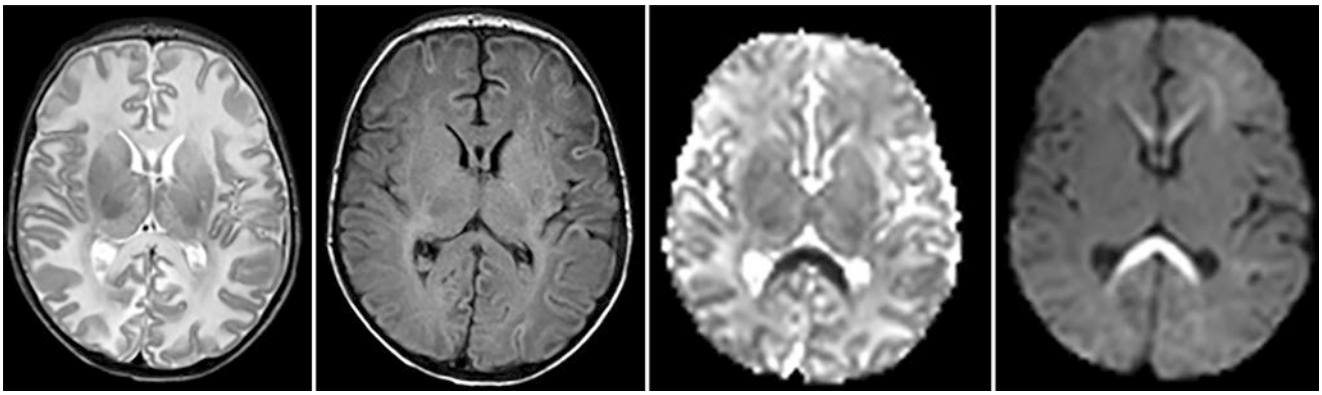

Fig. 33.2 Brain MRI (axial T2-weighted imaging, fluid attenuated inversion recovery [FLAIR], ADC map, and diffusion-weighted imaging) performed on day 10 of life in a term neonate with neonatal encephalopathy. T2-weighted imaging revealed diffuse extensive hyperintense signal throughout the white matter; in addition, there was abnormal hyperintense T2-signal in the deep gray matter, particularly in the putamen, globus pallidus, and caudate nucleus. There was loss of the normal cortical thickness in the frontal lobes bilaterally, as well as in the occipital lobes. There was extensive FLAIR hyperintense signal in the cortical gray matter, most pronounced in the right occipital medial cortex, as well as diffuse white matter hypointense signal on FLAIR imaging, predominantly in the frontal lobes and occipital lobes. Diffusion-weighted imaging revealed intense restricted diffusion along the corpus callosum, as well as less pronounced restriction scattered within the white matter in the left frontal lobe and bilateral occipital lobes. The abovementioned imaging features were characteristics of severe near-total injury

in the PLIC, abnormal signal intensities in the basal ganglia and thalami, loss of gray–white matter differentiation and cortical highlighting, as well as brainstem lesions. With respect to neonatal encephalopathy, the findings on brain MRI evolve over, so taking into account the postnatal age at the time of the scan is important for evaluating the exact extent of injury. The standard neonatal brain MRI protocol for neonatal encephalopathy includes conventional imaging (T1- and T2-weighted imaging) and diffusion-weighted imaging (DWI), as well as spectroscopy if available. The MRI findings on conventional imaging may be minimal within the first 24–48 h and thus may underestimate the full extent of an injury if analyzed too early. With respect to conventional imaging, usually, the loss of normal signal of PLIC is one of the first signs to appear, which may take up to 24–48 h, and which usually becomes more obvious in the first week of life [15]. Further signal abnormalities throughout the brain evolve between days 2 and 7 of life to become more obvious by the second week of life and then they gradually decrease over time and manifest as atrophy with or without cyst formation on conventional imaging [15]. Diffusion-weighted imaging (DWI) may help to detect brain injuries earlier than conventional imaging, as early as the second day of life [27], and they can be visualized for one week after the initial insult until the pseudonormalization of these images occurs around days 7–8 of life. Magnetic resonance (MR) spectroscopy may start to peak within the first 24 h of life, during which time conventional imaging may be

falsely negative [28]. MR spectroscopy peaks at days 2–4 of life after the initial insult [29] and is associated with an increase in lactates and a decrease in *N*-acetylaspartate (NAA) [30].

Outcome predictability using neuroimaging for neonatal encephalopathy is widely discussed in the literature. This information is crucially important for parent counseling. It also helps to guide therapeutic management in the early perinatal period. More extensive and multifocal lesions, especially when involving the basal ganglia, usually are associated with spastic/athetoid quadriplegic CP [31], intellectual disability, intractable epilepsy, and secondary microcephaly [32], which may be associated with feeding difficulties and therefore gastrostomy tube insertion [15]. In particular, the loss of a normal signal in PLIC is highly sensitive and specific of an abnormal neurodevelopmental outcome and CP in these near-term/term neonates [15, 33]. Watershed injuries are associated more often with cognitive deficits with or without motor impairments [31]. The NAA/Lac ratio in the basal ganglia was found to be the most accurate MR spectroscopic biomarker in predicting poor outcome [34]. The value of a brain MRI to predict neurodevelopmental prognosis did not appear to be affected by hypothermia treatment [32, 35].

Intraventricular Hemorrhage (IVH) and Periventricular Hemorrhagic Infarction (PVHI)

Germinal matrix hemorrhage (GMH) and intraventricular hemorrhage (IVH) describe the bleeding that is confined respectively to the germinal matrix or the ventricles [36, 37]; it is the most common form of intracranial hemorrhage in neonates, especially those who are premature. Periventricular hemorrhagic infarction (PVHI) describes a hemorrhage involving the periventricular white matter, which develops in 15% of all neonates with GMH–IVH [36]. GMH–IVH primarily affects premature neonates due to the fragility of their blood vessels in the germinal matrix and an immature cerebral autoregulation [37]. An inverse relationship exists between the incidence of GMH–IVH and gestational age and birth weight [37]. PVHI rises as a result of the bleeding in the germinal matrix and is not an extension of the intraventricular hemorrhage into the periventricular white matter [38]. The medullary veins, which are the veins draining the white matter, usually end in terminal veins within the germinal matrix. Hemorrhage into the germinal matrix may form a thrombus that can obstruct the medullary venous outflow, and venous infarction and/or necrosis may result as sequelae of this obstruction [36]. The region between the frontal horn of the lateral ventricle and the head of caudate nucleus is one of the most common regions where such an outflow obstruction can occur [36, 39]. Typically, both the GMH–IVH and PVHI develop within 96 h of birth (80–90%), and less than 20% already are present at birth [36]. Prematurity and low birth weight are among the major risk factors for the development of GMH–IVH and PVHI; other risk factors can include a low Apgar score, hypoxia, hypercapnia, and feto-maternal infection/chorioamnionitis [39], as well as Factor V Leiden or prothrombin gain of function mutations [40].

Although HUS is the most used brain imaging modality for evaluating the preterm brain, since it can be conveniently performed at the bedside, the quality of its images is highly operator-dependent, and the interpretations of its findings depend on the training and experience of the interpreter [41]. In this imaging, germinal matrix hemorrhage appears as an echogenic region at the level of the caudothalamic groove, and it should be distinguished from the mild echogenic appearance of the choroid plexus that might be present in the same location. HUS can easily detect intraventricular hemorrhage as an echogenicity extending from the caudothalamic groove into the ventricles, as cerebrospinal fluid–blood level or as casts of hemorrhage around the choroid plexus. Intraventricular hemorrhage can lead to ventricular dilatation due to the obstruction of the foramen of Monro (hydrocephalus) or due to an atrophy of the periventricular white matter [36]. PVHI appears as an echogenicity in the periventricular white matter, basal ganglia, or the thalamus [36].

MRI usually is performed at term-equivalent age (TEA) for preterm neonates. On T2-weighted imaging, germinal matrix hemorrhage may appear as darker linear regions in the caudothalamic regions or along the ependyma of the lateral ventricle, which is related to the presence of deoxyhemoglobin or hemosiderin. Susceptibility-weighted imaging (SWI) is a very sensitive MRI sequence for the diagnosis of GMH, detecting 42% of neonates with GMH compared to 25% when using HUS [39]. Hemorrhagic obstruction appears as a change in signal intensity that obstructs the foramen of Monro or cerebral aqueduct, or as septation. Venous infarction secondary to PVHI appears as a change in signal extending from the caudothalamic groove to the adjacent white matter and typically has a fan shape. Over time, PVHI will be replaced by a porencephalic cyst secondary to necrosis, and, therefore, a loss of parenchymal tissue [36].

The risk of CP increases in neonates with intraventricular hemorrhage, especially those with associated periventricular hemorrhagic infarction [39]. Other predictors of CP are the laterality of the periventricular hemorrhagic infarction, the number of involved territories, and the presence of midline shift [42]. The risk of CP in neonates with GMH and low-grade IVH does not differ from the risk of CP in neonates without detectable cerebral lesions [43].

Periventricular White Matter Injury (PWMI) or Periventricular Leukomalacia (PVL)

Periventricular white matter injuries are the most common lesions found in all children diagnosed later with CP (60% of all children with CP when children with ataxic CP are excluded) and around 90% of children born prematurely who later developed CP [44]. Typically, these lesions are linked to spastic CP, unilateral or bilateral [44].

HUS can easily detect cystic white matter injuries, but it has a very low sensitivity for detecting non-cystic white matter lesions compared to MRI and neuropathological studies [45]. Obviously, when cystic white matter injuries are present, the risk of CP is increased significantly (61% of all neonates with cystic PWMI developed CP) [46], regardless of whether it was unilateral or bilateral. Alternatively, non-cystic white matter lesions appear as inhomogeneous echogenicities on HUS [39, 46]. These lesions are very common in premature infants [47], and their presence in general—especially if they persist for ≥14 days [48] or if they persist until term-equivalent age [46]—has been associated with a higher risk of CP. Other HUS findings predicting the higher risk of CP include the presence of ventricular dilatation [48] or ventriculomegaly [49], as well as moderate to severe hydrocephaly [46]. Interestingly, the minimal HUS protocol that includes only one HUS during the first week and one at 36 weeks of gestation may miss these non-cystic echogenicities, since they may not be obvious early on and they may become less obvious around term-corrected age; thus, a weekly monitoring until term or discharge may be the best strategy for following their evolution [46].

The brain MRI typically performed at term-equivalent age (TEA) in preterm neonates is very sensitive for identifying white matter lesions that might be difficult to recognize on HUS. Typically, white matter abnormalities appear as diffuse, excessive, high-signal intensities on T2-weighted imaging, with increased apparent diffusion coefficient (ADC) values derived from diffusion-weighted imaging [47]. Other signs of white matter abnormalities also include a significant reduction in cerebral volumes in cortical gray matter, deep gray matter, and myelinated white matter, and a significant increase in cerebrospinal fluid volume [50]. Neuroimaging findings on the brain MRI predicting the higher risk of CP included the presence of asymmetric myelination on the MRI at term-equivalent age (a finding almost always associated with unilateral spastic CP) [51], decreased cerebral white matter without gliosis [52], cerebellar involvement with a particular involvement of the inferior portions of the cerebellar hemispheres and the vermis (a finding associated with the mixed motor pattern of spasticity, dystonia, and ataxia) [52], and a decreased size of the corpus callosum [53].

Children with bilateral spastic CP typically present with periventricular white matter injury and children with unilateral spastic CP typically display focal periventricular gliosis [44]. Other neuroimaging signs of PWMI that can be detected in childhood include enlarged ventricles, periventricular gliosis, decreased white matter volume, and a thinning of the corpus callosum [54].

Stroke

Perinatal stroke is a known risk factor for CP. Perinatal stroke can be classified into acute perinatal arterial ischemic stroke (PAIS) and presumed perinatal arterial ischemic stroke (PPAIS) [55].

Acute Perinatal Arterial Ischemic Stroke (PAIS)

Acute perinatal arterial ischemic stroke (PAIS) frequently occurs around the time of birth. Seizure is the most common presenting symptom (80–92% of cases) [55]. Other presenting symptoms include irritability, poor feeding, hypotonia, and apnea [55].

HUS and CT-scan can be used to evaluate PAIS, but MRI is the modality of choice for evaluating a suspected neonatal stroke. With respect to these cases, neuroimaging enables to estimate time of onset and provides insight about prognosis; it also permits an exclusion of other causes of brain injury that can mimic stroke presentation, such as encephalitis, hypoglycemia, and neonatal encephalopathy. One of the most sensitive MRI sequences for diagnosis in acute PAIS is diffusion-weighted imaging (DWI) with its associated apparent diffusion coefficient (ADC) maps. Ischemic lesions appear hyperintense on DWI and hypointense on ADC maps. The appearance of these lesions is likely secondary to the cytotoxic edema that results from the ischemia that leads to energy depletion [36]. Studies have found that ADC values eventually decrease for the first three days and then normalize during days 4–10 of life (pseudonormalization) [56]. T1-weighted and T2-weighted imaging are important for assessing different anatomical structures [55]. During the first six days of life, the infarcted cortex appears hyperintense on T1-weighted imaging and T2-weighted imaging, compared to the normal cortex. On the other hand, infarcted white matter appears initially hyperintense on T2-weighted imaging and mildly hyperintense on T1-weighted imaging; this hyperintensity persists for at least two to three weeks [36]. Another useful MRI sequence is susceptibility-weighted imaging (SWI), which can detect traces of blood in the brain parenchyma [55]. A magnetic resonance angiography (MRA) with or without contrast injection should be performed [36] since arterial anomalies are frequent in this population [57].

Adverse outcome depends on the occluded artery, and therefore on the subsequent size of the affected brain tissue. Studies have reported that PAIS leads to (mostly unilateral spastic) CP in 30–75% of infants with PAIS [58, 59]. Other consequences may include cognitive deficits, epilepsy, and language, visual, and behavioral issues in 50–75% of infants with PAIS [59]. The middle cerebral artery (MCA) was the most common artery affected by PAIS (90% of neonates with PAIS) [59]; the main MCA is the most common part of the MCA affected by PAIS (20–75% of neonates with PAIS), compared to the distal branches of the MCA [36, 59]. Posterior cerebral artery (PCA) and anterior cerebral artery (ACA) strokes are less common and only are reported in 10% of neonates with PAIS. All neonates with a main MCA stroke developed CP, while none of the neonates with cortical MCA developed CP and only 15–20% of neonates with other stroke subtypes (including anterior MCA, middle MCA branch, perforator MCA, ACA, or PCA) developed CP [60]. In the neonatal period, the corticospinal tracts involved at the level of the posterior limb of the internal capsule (PLIC) and the cerebral peduncle are associated with the development of CP [59, 61, 62]. Predictors for the development of CP are the involvement of the basal ganglia, thalami, and corticospinal tracts (CSTs) [59]; an association has not been found between an isolated thalamic involvement and CP development [59].

The recurrence risk of arterial ischemic stroke (AIS) in neonates is considered to be very low. This finding is based on a cohort of 215 neonates with arterial ischemic stroke (AIS), who were followed for an average of 3.5 years. During the follow-up period, 3.3% (7/215) developed a thromboembolic event and 4/7 (1.8%) had an AIS (venous sinus thrombosis, 2/7; and deep vein thrombosis of the leg, 1/7) [63]. Most of the cases with a recurrence of a second thromboembolic event had prothrombotic risk factors, including methylene tetrahydrofolate reductase (MTHFRT 667 T) mutation, elevated lipoprotein (a) [Lp(a)], elevated hyperhomocysteinemia, and/or protein C deficiency. Typically, the second event is triggered by underlying conditions such as congenital heart disease and immobilization, diarrhea and subsequent dehydration, mastoiditis, or moyamoya syndrome [63].

Presumed Perinatal Arterial Ischemic Stroke (PPAIS)

Presumed perinatal arterial ischemic stroke (PPAIS) is diagnosed after the neonatal period and retrospectively, it is presumed to have occurred perinatally. Patients are asymptomatic in the neonatal period, but later in life, they present with hemiplegia, focal hand weakness, or hand preference before they reach one year of age [36]. Typically, PPAIS is diagnosed by the middle of the first year of life or later [36]. Its diagnosis is confirmed by neuroimaging (typically a brain MRI) that demonstrates a chronic ischemic injury, such as the presence of encephalomalacia, gliosis, atrophy, or Wallerian degeneration [64]. The risk of recurrence is very low (<3%) [64].

Cerebral Venous Sinus Thrombosis (CVST)

Cerebral venous sinus thrombosis (CVST) is not uncommon in the neonatal period and studies have shown that neonates are among the most affected age group [65]. Several risk factors have been associated with neonatal CVST: that is, thrombotic risk factors (protein S and C deficiency, G20210A prothrombin mutation, Factor V Leiden mutation, antiphospholipid antibodies, infection, and polycythemia) [66], acute neonatal illness (infection, meningitis, or dehydration and other comorbidities like congenital heart disease), extracorporeal membrane oxygenation (ECMO) [36], and maternal risk factors (complicated and traumatic deliveries, maternal diabetes, and pre-eclampsia). A clinical presentation in the neonatal period is typically not specific and some neonates remain completely asymptomatic. A clinical presentation also may also include seizures, encephalopathy, or nonspecific symptoms like lethargy, poor feeding, or apnea [67].

The presence of an intraventricular hemorrhage in a full-term infant on HUS should raise the suspicion for cerebral venous sinus thrombosis, especially if the hemorrhage is associated with a unilateral thalamic hemorrhage. A decreased or absent flow in the affected sinus also can be indicated on Doppler ultrasonography [68]. When present, these findings should be confirmed by MRI and MR venography (MRV).

A head CT, especially a CT with venography, also can be used to confirm the diagnosis of CVST in neonates, although studies have shown that CT can miss lesions in 10–26% of patients [69] and can show false positive results in neonates [69]. Also, although the advantage of CT is its easy accessibility, the disadvantages of this method are the presence of ionizing radiation and the need for an intravenous administration of the contrast agent that carries the risk of allergic reaction, hypothyroidism, and contrast nephropathy. The "cord sign" is one of the signs suggesting of a CVST diagnosis that can be seen in the acute phase on a head CT without contrast; the venous sinuses appear hyperdense and expanded due to the presence of thrombosis [70]. The "dense triangle" sign may be seen on a head CT without contrast and represents a fresh clot in the superior sagittal sinus. In contrast, the "empty delta" or "empty triangle" sign may be seen on a head CT with contrast, which represents the opacification of the collateral veins in the superior sagittal sinus wall with a non-opacification of the clot inside the sinus [70].

Again, a brain MRI is considered the modality of choice for diagnosing neonatal CVST due to the absence of ion-

izing radiation and the higher sensitivity for detecting parenchymal lesions. However, to avoid an underdiagnosis or overdiagnosis, T1/T2-weighted imaging in multiple planes and MR venography are required [71]. Diffuse brain swelling can be identified on MRI as a result of venous hypertension and ischemia. Enlargement of the ventricles can be observed due to the impairment of venous outflow or as secondary to the obstruction associated with an intraventricular hemorrhage in deep venous infarction. Furthermore, MRI can identify focal edematous parenchymal lesions that involve the (mostly frontal and parietal) cortex and white matter in venous territories [36]. These lesions usually appear as a mixed pattern of signal changes on diffusion-weighted imaging and apparent diffusion coefficient maps, with regions of increased signals and regions of decreased signals. Neonatal CVST may be associated with hemorrhage within the lesions at the time of presentation.

In a Canadian registry, 77% of neonates with CVST had a normal neurological outcome at two years of age [72]. The adverse outcomes associated with CVST may include postnatal epilepsy, CP, visual deficits, cognitive impairments, post-hemorrhagic hydrocephalus requiring shunting, and death [36, 68]. The involvement of thrombosis in multiple cerebral venous sinuses and the presence of infarcted areas have been associated with long-term neurological deficits [73, 74]. CP has been found to occur mostly when CVST is associated with thalamic hemorrhage or neonatal encephalopathy [75].

Central Nervous System Infections

Bacterial Meningitis

Bacterial meningitis is a relatively common infection in the neonatal and infantile period, and several bacteria can cause it. The most common causative pathogen for early-onset (i.e., in the first seven days of life) bacterial meningitis in term newborns is Group B streptococcus (GBS), which accounts for 50% of cases, followed by *Escherichia coli* (*E. coli*) in 20% of cases, and *Listeria monocytogenes* (*L. monocytogenes*) in 5–10% of cases. Late-onset (i.e., between 7 and 28 days after birth) bacterial meningitis in term newborns usually is caused by GBS or *E. coli*. Very low birth weight (VLBW) infants are more susceptible to coagulase negative staphylococci, *E. coli*, *Enterobacter* (Fig. 33.3), and GBS; and although *Candida* is not a bacterium, it is another pathogen that has been found to cause meningitis in VLBW infants [76].

Neuroimaging usually is carried out for these infants to assess any potential complications that could be associated with bacterial meningitis, such as ventriculitis, brain abscess, cerebritis, cerebral infarcts, subdural empyema, hydrocephalus, cerebral venous sinus thrombosis, or intracranial hemorrhage [77–79]; in the absence of these complications, non-contrast CT and MRI usually are normal [77]. A brain MRI is the best neuroimaging modality to detect these complications associated with bacterial meningitis [77]. Eighty-one percent of infants confirmed to have bacterial meningitis presented with an abnormal brain MRI [78]. Leptomeningeal

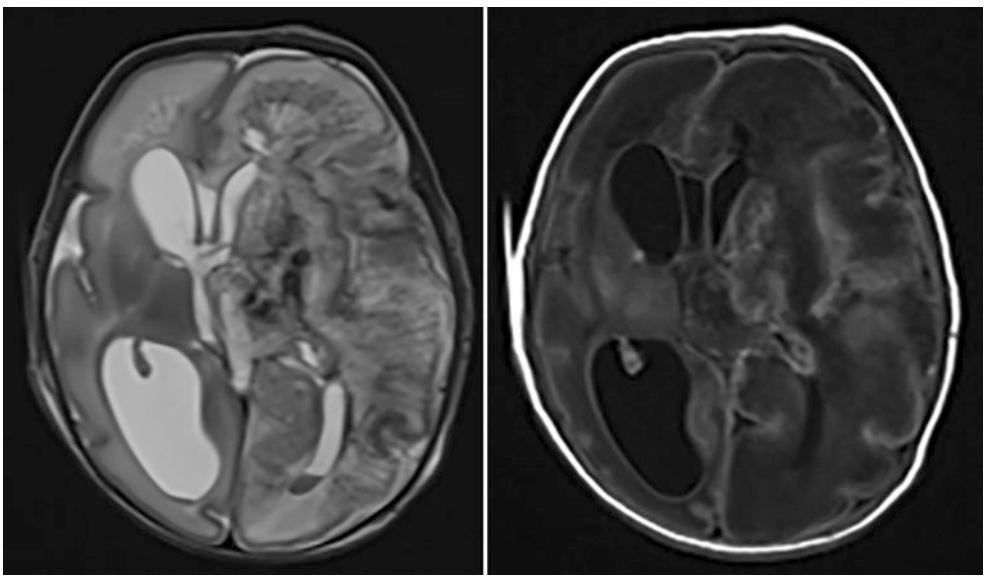

Fig. 33.3 Brain MRI (axial T1- and T2-weighted imaging) performed on day 10 of life in a 31-week female neonate with *Enterobacter meningitis* and bacteremia. Imaging demonstrated edema and swelling of the left cerebral hemisphere, resulting in significant rightward midline shift and asymmetric right lateral ventriculomegaly. Striated T1-hyperintense and T2-hypointense signals of the left hemisphere cortex, sparing the frontotemporal regions, denoting hemorrhages. Left thalamus markedly swollen, demonstrating hyperintense signal on T1- and T2-weighted imaging. Expected under-sulcation for degree of prematurity

enhancement is the most common neuroimaging finding for infants with bacterial meningitis, which appears as a contrast enhancement of the meninges and empyema of the ventricles [77, 78]. Although leptomeningeal enhancement is a classic finding, it is not specific for bacterial meningitis and can be present in other conditions such as leptomeningeal carcinomatosis, intracranial hypotension due to overshunting, and post-lumbar puncture [77]. On head CT, subdural empyema is visualized as hypodense, crescent-shaped extra-axial collections, whereas they appeared on T1-weighted imaging as isointense collections and on T2-weighted imaging as hyperintense collections, with diffusion restriction on diffusion-weighted imaging and low apparent diffusion coefficient values on ADC maps.

Bacterial meningitis can result in long-term complications, including CP, sensorineural hearing loss, cognitive impairments, global developmental delay, and epilepsy [80, 81]. CP is not an uncommon neurological complication of bacterial meningitis (6–15% of children with bacterial meningitis) [81]. The risk factors associated with CP are a longer duration of hospital admission, higher seizure frequency, intracranial hypertension, an increased protein level in the cerebrospinal fluid, and abnormal brain MRI [81]. Furthermore, neonates with GBS meningitis have a higher risk of neurological complications like subdural effusions and brain abscess, and those with *E. coli* meningitis have been found to have a higher risk of neurological complications such as subdural effusions, brain abscess, and hydrocephalus [79].

Herpes Simplex Virus (HSV)

An infection with the herpes simplex virus (HSV) can be transmitted as a transplacental infection that results in a congenital HSV infection (5–10% of HSV infections) or it can be transmitted by direct contact with the birth canal or with an individual with HSV skin lesions that results in a neonatal HSV infection (90–95% of HSV infections).

When acquired in utero, infection with HSV can cause microcephaly, encephalomalacia, ventricular enlargement, and intracranial calcifications [82] that can be visualized on neuroimaging [83].

Neonatal HSV infection is typically multifocal and tends to involve different brain regions that include white matter and cortical gray matter, as well as basal ganglia, and occasionally the brainstem and cerebellum [83]. The mortality and morbidity associated with neonatal HSV have been reduced dramatically with the utilization of acyclovir. Most (93%) of the neonates with disseminated HSV infection have developed normally, and 31% of the neonates with CNS disease also have developed normally at one year of age [84]. The risk factors associated with the increased morbidities of neonates with CNS disease were longer duration of the disease before starting therapy and presence of seizures [85]. Long-term neurodevelopmental abnormalities included microcephaly, spastic quadriplegia, persistent seizure disorder, and developmental delay [85]. Neuroimaging findings have been variable, depending on the timing of the imaging with respect to the course of the infection. In the first week of the infection, head CT may show edema appearing as hypodensities throughout the periventricular white matter and brain MRI may show hypointensities on T1-weighted imaging and hyperintensities on T2-weighted imaging [83]. Diffusion-weighted imaging may show areas of diffusion restriction in the periventricular white matter [86]. In addition, MR spectroscopy might be useful for demonstrating a decreased *N*-acetylaspartate (NAA), and possibly an elevated lactate [86]. Gyriform calcifications, cortical atrophy, and multicystic encephalomalacia become apparent only months later on neuroimaging [86].

Older children also may suffer from an HSV infection that involves the reactivation of the latent virus in the trigeminal sensory ganglion from a primary infection (e.g., gingivostomatitis), which results in the virus traveling along the trigeminal nerve and ventral leptomeninges typically to the inferior frontal lobes, infero-medial temporal lobes, and insular cortex. The initial CT-scan can be normal or it can show hypodensities in the inferior frontal and infero-medial temporal lobes, with asymmetric findings between the two hemispheres. A brain MRI can better characterize the affected anatomical structures, with signal changes classically appearing in the limbic system, insular cortex, cingulate gyrus, basal ganglia, and more rarely in the cortex of the parietal and occipital lobes. The lesions appear as an increased T2-weighted imaging signal intensity and a decreased T1-weighted imaging signal intensity; diffusion-weighted imaging indicates diffusion restriction, which has been found to be one of the early signs of this infection on neuroimaging. Furthermore, HSV-related encephalitis has been be associated with a petechial hemorrhage that appears as blooms of hyperintensities on a gradient recalled echo (GRE) sequence or as T1-shortening [86].

Kernicterus

Neonatal jaundice affects around 60–85% of neonates [87], whereas kernicterus or chronic bilirubin encephalopathy (i.e., the permanent complications of extreme hyperbilirubinemia) affects only a minimal percentage of neonates. The long-term sequelae of extreme hyperbilirubinemia, which usually become evident after one year of age, have been described as the classical tetrad: (1) increase in muscle tone, abnormal motor control, and abnormal involuntary movements; (2) vertical gaze palsy; (3) auditory processing disturbances with or without hearing loss; and (4) dysplasia of the enamel of deciduous teeth [88]. Patients with the motor pre-

dominant form can present with dyskinetic or athetoid CP. The most severe form also may be associated with the impairment of the voluntary movements and severe hypertonia [89].

Kernicterus is a clinical diagnosis that can be made even in the absence of neuroimaging findings such as T1- or T2-weighted imaging signal abnormalities [90] and/or in the absence of clinical neurological signs and symptoms of acute bilirubin encephalopathy in the neonatal period [91]. On brain MRI, kernicterus usually presents as a selective involvement of the globi pallidi, hippocampus, subthalamic nuclei, thalamus, hypothalamus, reticular portions of substantia nigra, as well as the dentate nuclei, roof nuclei, and Purkinje cells of the cerebellum [90]. In the acute phase, bilateral symmetrical high-signal intensities have been found on T1-weighted imaging, involving the globi pallidi and subthalamic nuclei, and to a lesser extent in the hippocampus and the dentate nucleus of the cerebellum [90]. Although these signal abnormalities usually are not associated with concomitant change on T2-weighted imaging, they could be associated with very mild hyperintense signals on this type of imaging [89, 90, 92]. Since it is important to differentiate these abnormal T1-weighted imaging high-signal intensities from a normal signal in these structures due to the normal myelination in term neonates [89, 90], the clinical context should help differentiate between the two. In the chronic phase, bilateral symmetrical high T2-weighted imaging signal abnormalities appear in the globi pallidi, subthalamic nuclei, hippocampus of the cerebrum, and in the substantia nigra and dentate nuclei of the cerebellum [93]. In this phase, the concomitant T1-weighted imaging signal can be variable. Data are limited about the findings associated with kernicterus on diffusion-weighted imaging. Although few studies have documented the absence of diffusion restriction in the globus pallidus and descending corticospinal tracts, specifically the cerebral peduncle [92], the presence of restriction diffusion in other anatomic structures that usually are involved in this pathological condition—including superior thalamic radiations, ventroanterior (VA) and ventrolateral (VL) nuclei of the thalamus, hippocampus, substantia nigra, subthalamic nucleus, superior cerebellar peduncle, pontine nuclei, and dentate nucleus of the cerebellum [94]—suggests a different time course of positive diffusion restriction among the different involved brain regions or the pathogenesis [94]. Interestingly, using MR spectroscopy, some studies have found a decreased *N*-acetylaspartate (NAA) to choline ratio, a decreased NAA to creatinine ratio, and an increased lactate to NAA ratio in the basal ganglia of neonates, who could potentially develop kernicterus [90]. Therefore, MR spectroscopy may be used in the neonatal period as a marker for infants who can develop the long-term sequelae of kernicterus in the future. In the neonatal period, conventional imaging also may indicate abnormalities in the basal ganglia and white matter in the neonates with kernicterus, who later may develop dyskinetic CP [95]; however, these changes are not always present early on [90].

Typical Neuroimaging Pattern of Some Prenatal Causes of CP

Congenital Brain Malformations

Congenital brain malformations are a less common cause of CP (12% of all patients with CP) [6]. These malformations are a group of brain defects or disorders that develop antenatally [96], which can be caused by genetic defects (either single gene defects or chromosomal abnormalities; Fig. 33.4) or environmental factors. Their associated variable clinical features include dysmorphic traits, severe intellectual delay, intractable seizures, developmental delay, and CP, and some of these features are incompatible with life and lead to death.

A brain MRI is a useful diagnostic tool for this group of disorders. Primary microcephaly and hydrocephaly are the most common types of these disorders found in children with CP due to brain malformations [6]. The brain malformations that are associated most commonly with CP range from holoprosencephaly, lissencephaly, pachygyria, and agenesis of the corpus callosum to posterior fossa malformation such as Joubert syndrome [97]; and these malformations are typically associated with a severe form of bilateral spastic CP [44]. Brain malformations such as cortical dysplasia, schizencephaly, (Fig. 33.5) and other focal malformations are associated with unilateral spastic CP. Cerebellar hypoplasia is associated with ataxic CP.

Interestingly, infants with non-cerebral malformations (2% of children with CP)—congenital heart disease, diaphragmatic hernia, and facial clefts—present more often with CP compared with the general population. Some researchers have speculated that the cause of CP in these cases is related to asphyxia after birth or anoxic insults in utero or in the perinatal or postnatal course [6].

Intrauterine Infections

Multiple infectious pathogens can cross the placenta and destroy the central nervous system (CNS). These agents include the classic TORCH (toxoplasmosis, rubella, cytomegalovirus [CMV], and herpes simplex virus [HSV]) infections, human immunodeficiency virus (HIV), varicella zoster virus (VZV), and lymphocytic choriomeningitis virus. Each of these intrauterine infections has been associated with an increased risk of CP (5–10% of all children with CP) [98]. The mechanisms through which these infectious pathogens cause injury in the neonatal brain can be attributed to the

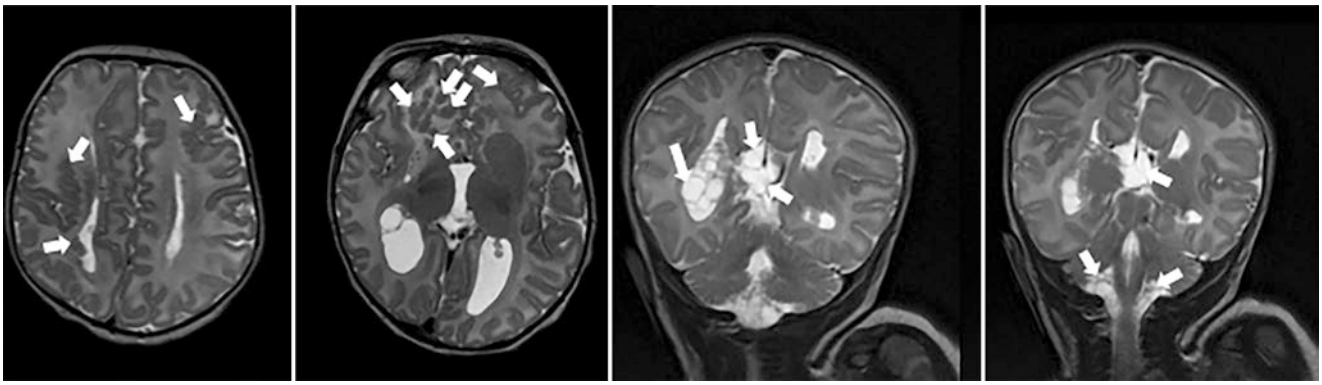

Fig. 33.4 Brain MRI (axial and coronal T1-weighted imaging) performed at one month of life in a female neonate presenting with right ptosis and smaller right palpebral fissure. Imaging displayed corpus callosum agenesis, extensive bilateral polymicrogyri with asymmetry in the size of the cerebral hemispheres, nodular gray matter heterotopias, white matter changes, interhemispheric cysts, right choroid plexus cysts, extra-axial posterior fossa cysts, and mild right cerebellar hypoplasia. The constellation of abovementioned findings (highlighted with arrows) is highly suggestive of Aicardi syndrome

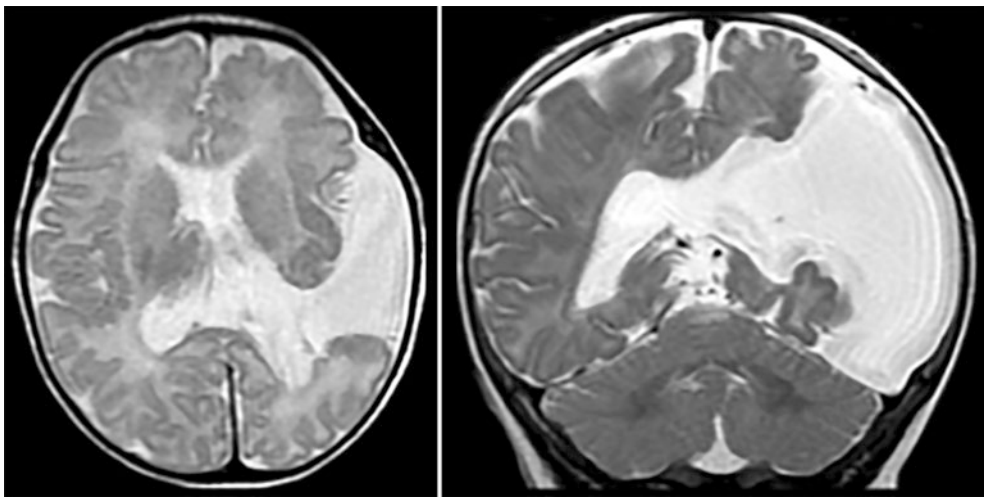

Fig. 33.5 Brain MRI (axial and coronal T2-weighted imaging) performed in a neonate. Imaging displayed typical picture of schizencephaly, with absence of the septum pellucidum and cleft in the brain parenchyma extending from cortical surface to the ventricle

direct inflammatory destructive effect or the indirect teratogenic effect of these different pathogens [96]. Following is a more detailed discussion of a few of these intrauterine infections.

Congenital CMV (cCMV)

Congenital CMV (cCMV) is the most common and serious congenital infection. Its incidence rate varies between 0.2 and 2.5% of neonates [99]. Neurological complications develop only in a small percentage of infants with a congenital CMV infection. During the neonatal period, the risk factors for long-term neurological disabilities include chorioretinitis, microcephaly, and early neurologic abnormalities in the neonatal period [100].

The neuroradiological features of cCMV include a wide spectrum of brain anomalies such as migrational defects (e.g., lissencephaly), intracerebral calcifications, white matter abnormalities (e.g., parenchymal cystic changes and delays in myelination), cerebral and cerebellar atrophies (e.g., hypoplastic corpus callosum), periventricular/subependymal cysts, and meningoencephalitis [97]. Some of these neuroimaging abnormalities (the presence of discrete or extensive periventricular calcifications, lenticulostriate vasculopathy, and/or brain atrophy) have been associated more frequently with the development of adverse neurological sequelae including (mostly spastic or dyskinetic) CP [101]. The risk of CP among the survivors of neonatal symptomatic CMV ranges between 12 and 50% [98]. These findings can be detected by HUS, head CT, and brain MRI.

HUS already may be able to detect the different brain abnormalities associated with congenital CMV infection, including increased periventricular abnormalities, ventriculomegaly, intracranial calcifications, intraventricular adhesions, thalamic hyperechogenicity, mega-cisterna magna, lissencephaly, vermian defects, and cerebellar cysts [102].

A head CT is especially helpful for detecting intracranial calcifications, compared to other modalities. A head CT also may indicate white matter abnormalities, ventriculomegaly, neural migration disorder defects, brain atrophy, and destructive encephalopathy [103].

Brain MRIs indicate the same abnormalities. In addition, MRI is particularly sensitive for detecting disorders of neuronal migration, parenchymal cystic changes, delays in myelination, and cerebellar hypoplasia that could be missed by other neuroimaging modalities [100].

Congenital Toxoplasmosis

The severity of the brain lesions associated with congenital toxoplasmosis depends on the timing of the infection and can range from mild atrophy and minimal periventricular calcifications to severe diffuse large parenchymal calcifications [104]. Earlier infections during gestation appear to be correlated with larger calcifications [105].

Neuroimaging signs of congenital toxoplasmosis are multiple and include the dilatation of the posterior horns of the lateral ventricle and multiple echogenic nodular foci that correspond to the foci of the calcifications in periventricular white matter injury, cortex, and basal ganglia [106]. Both of these abnormalities can be detected by antenatal ultrasounds [107]. Researchers also have reported dysgenesis of the corpus callosum [106].

Brain MRI may show diffuse hyperintensities throughout the brain parenchyma in T2-FLAIR (fluid attenuated inversion recovery) imaging [107]. Other abnormalities that have been observed are parenchymal cystic changes [106], microcephaly, macrocephaly, hydrocephaly, and brain atrophy [83], as well as microphthalmia.

After the implementation of the current treatment regimen, the adverse outcome of congenital toxoplasmosis was significantly reduced. However, if left untreated, symptomatic congenital toxoplasmosis remains associated with an unfavorable outcome, and even infants with systemic toxoplasmosis (without CNS disease) present with a poor neurological outcome. Spastic CP was found in 76% of neonates with neurological toxoplasmosis and in 58% of neonates with systemic toxoplasmosis [96]. Although no studies reported the prognostic value of each neuroimaging finding, a more favorable prognosis was reported when the cerebral parenchymal lesions were not associated with ventriculomegaly [108].

Typical Neuroimaging Pattern of Some Postnatal Causes of CP

Non-Accidental Head Injuries (NAHI)

Non-accidental head injury (NAHI) or abusive head trauma (AHT) is defined as an injury to the skull or intracranial structures of an infant younger than five years due to an intentional abrupt direct impact, compressing and penetrating injuries, and/or violent shaking injuries. This definition does not include injuries resulting from neglect, gunshot wounds, stab wounds, or penetrating trauma [109]. In the pediatric population, NAHI is associated with high rates of mortality and morbidities [110]. Morbidities include CP, cognitive and behavioral disturbances, blindness, and epilepsy [109]. The typical age at presentation of NAHI varied from 3 weeks to 11 months [109]. The clinical factors associated with poor long-term neurodevelopmental outcome included low initial Glasgow Coma Scale (GCS), bilateral retinal hemorrhage, and skull fracture at presentation, as well as poor head growth after the injury [109].

A head CT is an important neuroimaging modality for evaluating NAHI and it is the best imaging modality for detecting skull fracture and cerebral contusions. A linear parietal skull fracture is the most commonly observed fracture associated with non-accidental injury [111]. Fractures that are multiple, depressed, diastatic, or located in the occipital bones also are suspicious for NAHI [111]. Craniocerebral erosions or leptomeningeal cysts can be present in association with the linear skull fracture of the parietal bone in children <3 years old. Subdural hematomas are another classic features, which usually are bilateral and symmetrical. Brain MRI is useful for detecting parenchymal lacerations, diffuse axonal injuries, and microhemorrhages, and for determining the timing of the injury [111]; and MRI is the modality of choice for determining the prognosis of infants with NAHI. Parenchymal lacerations—resulting from the shearing of the white matter secondary to shaking and/or an impact trauma that spares the gray matter—are characteristic in infants younger than five months, who present with NAHI [111]. The preferential involvement of the subcortical white matter is explained by the different consistency between the gyral gray matter and the unmyelinated subcortical white matter, which makes the latter more susceptible to shearing injury [111]. Diffuse axonal injuries resulting from rotational force appear as diffusion restriction [111]. Microhemorrhages that might be associated with diffuse axonal injuries are present at characteristic locations that include the parasagittal gray–white matter interface, corpus callosum, internal capsule, and brainstem [111]. Subpial hemorrhages also can be found in NAHI [111]. Diffusion-weighted imaging has been very useful for detecting early ischemic injuries. In addition, MR spectroscopy has indicated low ratios of *N*-acetylaspartate/choline, *N*-acetylaspartate/creatinine, and *N*-acetylaspartate/lactates in infants with poor prognosis [112].

CT and/or MRI signs have found that the presence of (diffuse) intraparenchymal lesions is associated with poor neurodevelopmental outcome [109]. The severity of the motor and cognitive impairment depends on the extent of these parenchymal lesions [109].

End-of-Chapter Summary/Bullet Points

- CP is non-progressive disorder of motor development. It can be secondary to prenatal, perinatal, or postnatal causes. Neuroimaging is essential in the work-up for children with CP. Neuroimaging findings depend on the underlying CP etiology.
- The pattern of injury associated with neonatal encephalopathy can be subdivided into three main categories: basal ganglia injury pattern, watershed pattern, and near-total injury pattern. The pattern of injury depends on the severity and duration of the event leading to the asphyxia and the timing of the neuroimaging. The MRI sequences recommended for evaluating neonatal encephalopathy include at least conventional T1-weighted imaging, T2-weighted imaging, diffusion-weighted imaging, and MR spectroscopy. The pattern indicated on MRI depends on the timing of the neuroimaging—for example, diffusion-weighted imaging is more useful early on and conventional imaging works best later on. An abnormal signal within the PLIC is a predictor of abnormal motor outcome in infants with neonatal encephalopathy.
- Intraventricular hemorrhage (IVH) is the most common intracranial bleeding in neonates, especially preterm neonates, and it can be detected easily with HUS as an echogenicity extending from the caudothalamic groove into the ventricles. Periventricular hemorrhagic infarction (PVHI) is a hemorrhage involving the periventricular white matter, and it appears on HUS as an echogenicity in the periventricular white matter, basal ganglia, or the thalamus. Susceptibility-weighted imaging (SWI) is the most sensitive MRI sequence for detecting both abnormalities. The risk factors for CP in neonates with PVHI are the unilateral location, midline shift, and number of involved territories.
- Periventricular white matter injuries (PWMI) are the most common lesions found in children diagnosed with CP, if ataxic CP is excluded. Head ultrasound can easily detect cystic PWMI, but it is less sensitive to non-cystic PWMI. A brain MRI at term-equivalent age (TEA) is very sensitive for detecting non-cystic PWMI, which is indicated by increased signal intensities on T2-weighted imaging. On brain MRI, the presence of asymmetric myelination, reduced cerebral white matter without gliosis, involvement of the inferior portion of the cerebellar hemisphere and vermis, and reduced corpus callosum size are predictors for CP development.
- Acute perinatal arterial ischemic stroke (PAIS) occurs around the time of birth. A brain MRI with diffusion-weighted imaging is the modality of choice to determine the time of PAIS onset and to predict its prognosis. Conventional T1-weighted imaging and T2-weighted imaging are important for detecting the anatomical structures involved. PAIS usually is associated with unilateral spastic CP, and the severity of this adverse outcome depends on the size of the affected brain tissue.
- Cerebral venous sinus thrombosis (CVST) is relatively common in the neonatal period. CVST usually is associated with risk factors such as prothrombotic risk factors, acute neonatal illness, comorbidities like congenital heart disease, and extracorporeal membrane oxygenation (ECMO). Conventional MRI and MR venography (MRV) are the modalities of choice. Most neonates with CVST had a normal neurological outcome at two years of age. CP is indicated if CVST is associated with thalamic hemorrhage or neonatal encephalopathy.
- Bacterial meningitis is a common infection in the neonatal period, but CP is an uncommon complication of bacterial meningitis. A brain MRI can be normal or show abnormalities depending on the causative organism of meningitis. The risk factors for CP are a long hospital admission, high protein level in the cerebrospinal fluid, high seizure frequency at presentation, and an abnormal brain MRI.
- Herpes simplex virus (HSV) encephalitis can be congenital (transplacental transmission, rare) or present in the neonatal period (most frequent) due to direct contact with the birth canal or with an individual with HSV skin lesions. Neuroimaging findings can include multifocal lesions of T2-increased signal intensities with diffusion restriction and petechial hemorrhage. CP is one of the long-term complications of HSV encephalitis.
- Kernicterus is an avoidable complication of neonatal hyperbilirubinemia. It is a clinical diagnosis. A brain MRI may indicate an increased T1-signal intensity in the acute phase and an increased T2-signal in the chronic phase in specific anatomical structures, typically the globi pallidi.
- Congenital cytomegalovirus infection (cCMV) is the most common congenital infection. Neuroimaging can identify the different abnormalities associated with cCMV, including brain migrational defects, intracranial calcifications, and white matter abnormalities. The presence of discrete or extensive calcifications, lenticulostriate vasculopathy, and/or brain atrophy on MRI has been associated with an increased risk of CP.
- The severity of the brain lesions associated with congenital toxoplasmosis depends on the timing of the infection. Neuroimaging findings are T2-hyperintensities throughout the brain, parenchymal cystic changes, intracranial calcifications, microcephaly, macrocephaly, and brain atrophy. Neurological toxoplasmosis carries a higher risk for the development of CP compared to systemic toxoplasmosis.

- Congenital brain malformations are not a common cause of CP. A brain MRI is an essential tool to diagnose brain malformations and to predict their prognosis, especially the risk of CP. Non-cerebral malformations such as congenital heart disease also may be associated with an increased risk of CP.
- Non-accidental head injuries can be associated with CP. Neuroimaging findings associated with poor neurodevelopmental outcome include the diffuse intraparenchymal lesions that have been detected by CT and/or MRI after the head trauma.

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Multiple Choice Questions and Answers

Question 1: You have seen a 24-month-old girl in your office for developmental delay. Her mom told you that she started sitting independently at 11 months and that she started walking at 20 months. Her fine motor development and her speech are normal. Her mom told you that she was born at 28 weeks of gestation, that she was admitted to NICU for 8 weeks and that she was on a ventilator for a long period of time. You found that she had a grade 2 IVH when she was in the NICU, but on discharge, her HUS was normal with no evidence of any IVH. An examination showed spasticity in the lower extremities with increased deep tendon reflexes at 3+ and non-sustained clonus. What is the most likely diagnosis for this girl:

a. Idiopathic toe walking
b. Diplegic cerebral palsy
c. Tetraplegic cerebral palsy
d. Early signs of hereditary spastic paraparesis

Correct answer: b

Question 2: What are the typical MRI findings for the previous scenario:

a. Periventricular calcifications
b. Bilateral schizencephaly
c. Periventricular white matter injury
d. Bilateral subdural hematoma

Correct answer: c

Question 3: In the previous scenario, the mom of the little girl asked you why her legs are predominantly involved rather than her arms. What would be your response?

a. No clear explanation exists for such a clinical presentation of cerebral palsy
b. Because the corticospinal white matter pathway derived from the cortical area corresponding to the legs is further posterior compared to the pathway derived from the cortical area corresponding to the arms, and because a periventricular white matter injury is usually more severe posteriorly
c. Because the intraventricular hemorrhage was more severe in the area corresponding to the legs than the cortical area corresponding to the arms
d. Because the corticospinal white matter pathway derived from the cortical area corresponding to the legs is further anterior compared to the pathway derived from the cortical area corresponding to the arms, and because a periventricular white matter injury is usually more severe anteriorly

Correct answer: b

Question 4: You have been asked to evaluate a term neonate in the neonatal intensive care unit presenting with seizures since 7 h of life. He was loaded with phenobarbital and has been started on a maintenance dose. When reviewing his chart, you found that his antenatal history was uneventful except that there was a history of cord prolapse. The baby was born flat and required positive pressure ventilation, intubation, epinephrine and cardiac massage. On examination, the baby was intubated and was severely hypotonic. He had no corneal reflex and a weak gag reflex. The NICU team asked your advice about a neuroimaging evaluation for this case.

a. You would obtain an urgent head CT to look for evidence of any intracranial hemorrhage
b. You would ask for an urgent HUS to look for intraventricular hemorrhage
c. You would ask for a brain MRI, including T1-weighted imaging, T2-weighted imaging, diffusion-weighted imaging, and MR spectroscopy
d. No need for neuroimaging, since you would consult palliative care

Correct answer: c

Question 5: You received a referral from a community pediatrician for a 4-year-old boy who recently had immigrated with his parents, and it was noticed that he had difficulty holding crayons and coloring at his daycare. You cannot communicate with the parents because of a language barrier, and you request an interpreter. While waiting for the interpreter, you examine the boy, and find microcephaly (head circumference < 3rd percentile), and a cranial nerve examination shows lower facial weakness with drooling. A motor examination shows spasticity of 4 extremities (upper extremities > lower extremities) and deep tendon reflexes of 3+ throughout with sustained clonus. You learn from the com-

munity pediatrician that the boy's development did not show regression. In addition, the boy had extensive metabolic and genetic work-up that was negative.

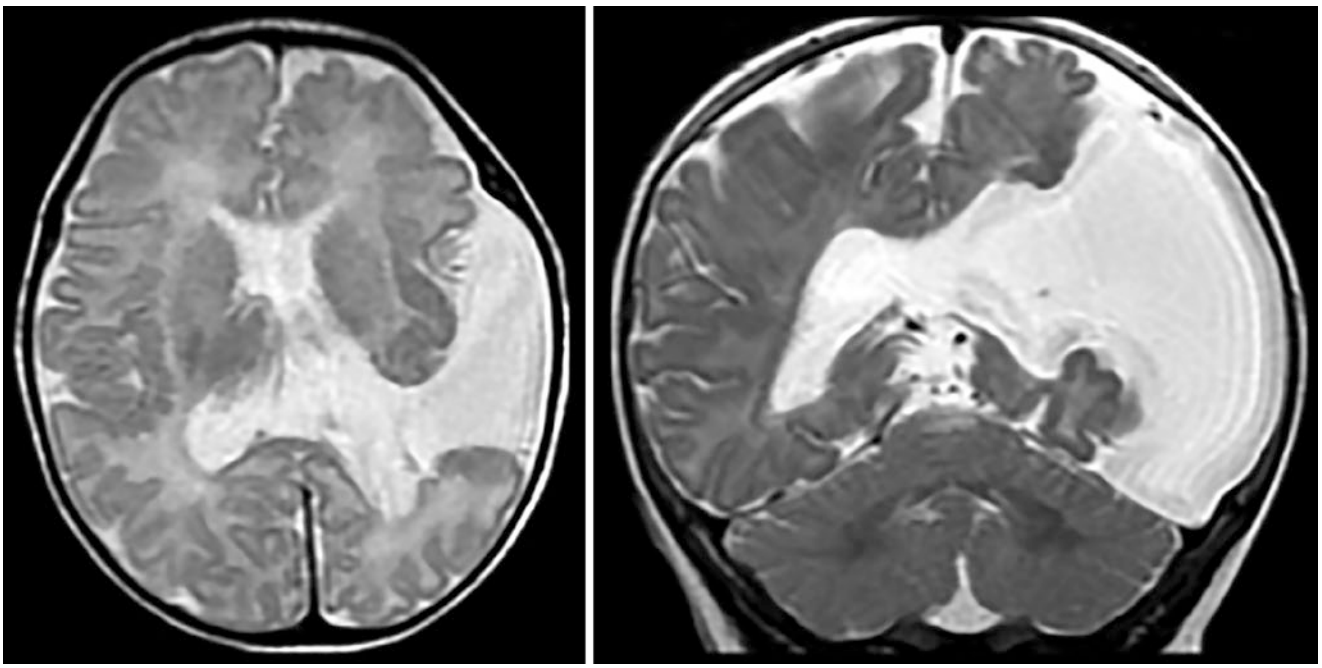
All of the following could be potential etiologies for his primary disorder, except for:

a. Perinatal hypoxic-ischemic brain injury in term infants (prolonged partial asphyxia)
b. Congenital infections (TORCH infections)
c. Bilateral early prenatal stroke, resulting in schizencephaly or polymicrogyria
d. Chronic bilirubin encephalopathy

Correct answer: d

a, b and c can be potential causes for the arm-dominant spastic quadriparetic CP of this boy

Question 6: In your office, you evaluated a 15-month-old boy, who presented with global developmental delay and seizures that localized to the right side of his body. On examination, he presented with microcephaly, and his muscle tone was increased overall, but more on the right side compared to the left. Here is his magnetic resonance imaging that you just obtained (Fig. 33.5).



What is the likely explanation of this presentation?

a. Left-sided open-lip schizencephaly
b. Congenital CMV infection
c. Hydrocephalus
d. Parenchymal cyst
e. Hemimegalencephaly

Correct answer: a

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Neuropathology of Cerebral Palsy

34

Harvey B. Sarnat

Learning Objectives

- The reader should be able to understand some of the underlying principles of neuropathology and immunohistochemistry.
- The learner will be able to understand the aetiologies and the underlying pathologies associated with CP

Highlights

- Neuropathology provides a dimension to enhance understanding of the aetiology and pathogenesis of cerebral palsy that compliments clinical, neuroimaging and genetic data. Modern neuropathology supplements simple histological stains with specific immunocytochemical markers that can denote cellular lineage and maturation of neurons and glial.
- The spectrum of neuropathological changes in the brains of infants and children with cerebral palsy is as broad and varied as are the aetiologies, but the most frequent lesion remains subcortical white matter atrophy and gliosis.
- Perinatal hypoxic/ischaemic encephalopathy remains a major cause of cerebral palsy, but cerebral malformations are increasingly recognized as more frequent than previously thought.

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Introduction

Aetiology and Pathogenesis

Cerebral palsy is neither an aetiological diagnosis nor does it designate pathogenesis or mechanisms of brain or spinal cord damage. *Aetiology* signifies the fundamental cause of pathological changes, such as a genetic mutation, an epigenetic factor such as foetal exposure to an organism causing neonatal meningitis. Pathogenesis, by contrast, explains the mechanism by which normal developmental processes are interrupted or altered, such as arrested neuronogenesis or neuroblast migration. The key to understanding cerebral malformations is neuroembryology, foetal maturation of the nervous system and developmental neuropathology closely linked to timing of onset of genetic expression or time of an acquired insult in relation to nervous system development at that moment [1, 2]. Magnetic resonance imaging (MRI) studies of term neonates with cerebral palsy, even decades ago, supported neuropathological findings that implicated prenatal factors more than intrapartum asphyxia or other acute perinatal events [3]; more recent neuroimaging studies confirm this conclusion. Modern genetic studies also are valuable in differentiating aetiologies of cerebral palsy [4].

Because the aetiologies of cerebral palsy are so diverse, neuropathological findings are equally diverse. Lesions include loss of upper motor neurons from layer 5 of the cerebral cortex, especially frontal lobe, gliosis, microglial activation and inflammatory cells in the cortex and subcortical white matter, and deep subcortical white matter lesions. The latter is the most frequent finding in most cases and is designated periventricular leukomalacia (PVL) in neuroimaging studies. PVL can extend into the posterior limb of the internal capsule and impair or cause loss of corticospinal tract fibres with secondary loss of their large pyramidal neurons in the cortex. White matter injury demonstrated by ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) is confirmed neuropathologically. However, there are

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many other lesions that can mimic PVL in imaging studies, so that caution is needed in interpretation [5].

Epigenetics no longer is regarded as simply as any insult to the body or brain that is not a primary genetic mutation or chromosomopathy. Some neurotoxins and teratogens of the foetal environment are now documented to be capable of altering the DNA of foetal progenitor cells and thus cause a genetic mutation as an important part of their pathogenesis. An example is the foetal alcohol spectrum disorder, with postnatal clinical correlates of global developmental delay, sometimes autism, sometimes epilepsy and often a degree of cerebral palsy with either spastic or hypotonic diparesis. In this disorder induced by foetal exposure to maternally ingested alcoholic beverages, the foetal DNA methylation is altered in various patterns and affects RNA transcription and protein synthesis [6, 7]. Abnormal DNA methylation also forms a basis for classification of brain tumours [8]. It is likely associated with many other foetal neurotoxins and drugs ingested by the mother and to result in postnatal cerebral palsy and other neurological defects. Foetal effects from teratogenic toxins may be single exposures, as with a one-time maternal drug ingestion before she knew she was pregnant, to exposure over an extended time period of different developmental processes, as with congenital infections or repeated maternal drug abuse throughout gestation.

Semantics

Semantics do matter! The precision by which one expresses one's self implies the precision of scientific or medical research studies that one conducts and from which the reader tacitly infers confidence in the author's conclusions [9, 10]. Terminologies may differ amongst various authors and especially between different medical specialty disciplines, sometimes resulting in ambiguity rather than clear communication. 'Cerebral palsy' is a nineteenth century designation of any non-progressive and non-transitory motor deficit dating from prenatal life, birth or early infancy. It is not, therefore, applied to acquired hemiplegia due to stroke in adult life. The term *cerebral palsy* is broad in encompassing many types of motor disturbances with focal, unilateral or bilateral clinical manifestations. It can be further specified with adjective descriptors such as *ataxic*, *dystonic* or *athetotic*. It remains a popular term amongst developmental paediatricians and orthopaedists in particular, but has fallen out of favour amongst paediatric neurologists who tend to prefer more precise descriptors such as *spastic diplegia* (or *diparesis* if the motor deficit is less dense). At times, more neuroanatomical terminology is applied, such as corticospinal tract (CST) deficit, but this also can be ambiguous at times because the cerebellum and basal ganglia have their major expression via the CST, as cerebellospinal, striatospinal and pallidospinal tracts do not exist in humans. Small bulbospinal pathways do mediate some cerebellar functions in infancy before the CST is fully mature in terms of terminal axonal sprouting, synaptogenesis and myelination, such as the rubrospinal, olivospinal and vestibulospinal tracts [11]; cerebellar functions involve not just the cerebellar cortex but several circuits or networks such as *Guillain-Mollaret triangle* (dentato-rubro-olivo-cerebellar cortical circuit) [12]. Clinically, gross and fine motor deficits also may be distinguished with neuroanatomical correlates. Despite my personal reservations about the label *cerebral palsy*, I will use this term here for consistency with authors of other chapters to avoid ambiguity.

Another example of a lack of semantic precision is the term 'quadriplegia', an incongruity that attempts to link a Latin prefix to a Greek root. Diplegia, consistently Greek in origin, is more acceptable and does not necessarily imply that the involvement of the two sides of the body are symmetrical or equal. Tetraplegia, also all Greek, implies a cervical spinal cord injury that spares the corticobulbar tracts to cranial nerve innervated muscles and paraplegia (also all Greek) is due to a lower spinal cord injury that spares the cervical spinal segments that innervate the upper limbs. The proposal that quadriplegia and diplegia distinguish whether the upper or lower extremities are predominantly involved is not universally understood or accepted and is a poor reason to perpetuate a semantic error.

Yet another semantic imprecision is the use of *ectopic* and *heterotopic* interchangeably; in morphogenesis and ectopic cell is one that is displaced outside its organ of origin, whereas an *heterotopic* cell is displaced within its organ of origin. If the brain is considered the organ of origin, a neuron isolated within the leptomeninges is ectopic; a neuron neuropathologically displaced to the deep subcortical white matter (centrum semiovale) is heterotopic; ectopic neurons cannot occur within the brain by teratological definition [9, 10]. *Heterotopia* is already a plural form of this Greek derivative, the singular form being heterotopion, a parallel structure to *mitochondrion/mitochondria* or *criterion/criteria*. 'Heterotopias' has a redundant final 's' and thus is incorrect English usage, though the Romance languages (e.g. French, Spanish, Italian) convert such 'foreign' Latin and Greek terms to correspond to the grammar of those languages. The terms 'periventricular leukomalacia' and 'white matter injury' have been used interchangeably and synonymously in the literature, but they may not be identical and, if used correctly, they may convey greater precision of the nature of the lesion in a couple of words; neuropathological distinctions are not always evident in MRI of foetuses and preterm neonates [13].

Traditional and Modern Neuropathological Examination

Macroscopic or gross neuropathological examination at autopsy began in the mid-nineteenth century by Rudolph Virchow, who initiated a systematic inspection of the brain and spinal cord and described their appearance. This practice continues, including normal and abnormal convolutions and dorsal viewing of the corpus callosum within the interhemispheric fissure. It also includes the macroscopic appearance of internal structures revealed by sections of the brain usually cut in the transverse (coronal) plane but sometimes cut horizontally (axially) to correspond to imaging findings or sagittally for special anomalies such as Chiari malformations. Further development of neuropathology is best understood from a chronological historical perspective.

Traditional neuropathological examination included not only the macroscopic findings that could be discerned by the naked eye, but also microscopic anatomy using a variety of general tissue stains, the most popular in the nervous system being haematoxylin-eosin (H&E) or Nissl stains such as cresyl violet. But even in the late nineteenth century special purpose stains began to appear that demonstrated subcellular structures not revealed by general histological stains. The most important was silver (and sometimes mercury) impregnation of neuronal and glial processes that enabled interpretation of the organization and development of structures of the nervous system at the microscopic level. This impregnation technique was first developed by Camille Golgi of Italy, who illustrated the first internal brain structures with the olfactory bulb [14]. Insightful interpretation of Golgi impregnations, including the concept of individual nerve cells connected by synaptic connections and descriptions of the development of the foetal CNS, was made by the still valid classical studies of Santiago Ramón y Cajal of Spain [15, 16], and continuing into the late twentieth century by Miguel Marín-Padilla [17]. Golgi and Ramón and Cajal were jointly awarded the Nobel Prize in Physiology and Medicine in 1906. Transmission and also scanning electron microscopy began in the mid-twentieth century, for the first time enabling detailed subcellular structural examination of cellular organelles, synapses and membranes below the resolution of the light microscope.

The modern period of neuropathology began in the last quarter of the twentieth century, first with the development of histochemistry, such as the demonstration of specific mitochondrial enzymatic activities in brain and in muscle biopsies (but which required frozen sections of unfixed tissue) and the demonstration of specific metabolic products within the cell, both normal and abnormal storage. The first such metabolic stain dates from the early twentieth century and was the periodic acid-Schiff reaction to identify glycogen and polysaccharides, both intracellular and in the extracellular matrix. A technique to demonstrate myelin in paraffin-embedded tissue sections was described by Klüver and Barrera [18] and Paul Yakovlev provided the first comprehensive survey of myelination sequences in the human brain from birth to old age [19], followed by other investigators who confirmed and expanded his findings [20–22]. In the 1960s and 1970s fluorochromic stains such as acridine orange (AO) that require a fluorescence microscope demonstrated nucleic acids with different colour perception of DNA and ribosomal RNA that detected neuronal maturation in the developing brain as a marker of neurotransmitter biosynthesis [23], neurones altered by chromatolysis from hypoxic-ischaemic encephalopathy [24], or degenerating motor neurones in spinal muscular atrophy [25]. AO is a more sensitive measure than cytological changes seen with H&E, but is not as sensitive or quantitative as microarrays of mRNA analysis that evolved in the early twenty-first century [26].

In the 1980s the first immunocytochemical antibodies applied to paraffin-embedded tissue sections were introduced. The specificity of antibodies enabled the demonstration of many molecules within cells. GABAergic inhibitory interneurons can be distinguished by calcium-binding compounds (e.g. calretinin; parvalbumin; calbindin D28k) not found in glutamatergic neurons [27–29]. Immunocytochemistry also provided a means of demonstrating neuronal maturation and synaptogenesis in tissue sections, both in normal brain development and in malformations and pathological conditions in which there might be an induced maturation delay [27, 28]. MRI can demonstrate timing of myelination in white matter tracts, but cannot yet detect sequences of synaptogenesis or the expression of mature neuronal and glial proteins.

One of the most frequent and most serious of the multiple lesions that also cause cerebral palsy is epilepsy, especially severe infantile and juvenile epilepsies such as infantile (epileptic) spasms (West syndrome), Ohtahara syndrome and Lennox-Gastaut syndrome, as well as focal and generalized seizures. Abnormal synaptic circuitry and networks is the basis for epilepsy. Immunocytochemical reactivity in tissue sections with antibodies against synaptic vesicular membranes, such as synaptophysin, synaptotagmin, synaptobrevin and SNAP-25. Synaptophysin is the glycoprotein most frequently used in neuropathology in the diagnosis of tumours and adult neurodegenerative diseases, but also is a reliable method of demonstrating the sequence of synaptogenesis in the cerebral cortex, hippocampus, basal ganglia, cerebellar system and other regions of the CNS [12, 30–32]. Delayed, arrested and precocious synaptogenesis, as well as abnormal distributions of synapses also can be demonstrated by synaptophysin [2, 12, 30, 32, 33]. Specific immunocytochemical markers can denote individual neuronal maturation at various stages during development [27, 28]. Immunoreactivity in tissue sections

to specific neurotransmitters and their enzymes of biosynthesis and degradation, and to specific gene transcription products also are now available, that extends the diagnostic capability and value of neuropathology in children with cerebral palsy, in both surgical resections for epilepsy and at autopsy. Unfortunately, these resources or neuropathologists with interest are not available in all laboratories and often the neuropathology of the year 1920 is performed with H&E-stained sections only.

Just as electroencephalography (EEG) has progressed from 8-channel paper recordings of 45 min to computerized continuous monitoring with 32 channels, neuroimaging has progressed from pneumoencephalography and angiography to computed tomography to basic sequences of magnetic resonance imaging (MRI) to now increasing capability of functional MRI sequences, and medical genetics has progressed from identifying Mendelian traits to identification of a myriad of genetic mutation and whole exome and entire genome sequencing, so has neuropathology progressed from simple descriptive gross and microscopic morphology to precise tissue localization of metabolic products, distinction of neuronal types and transmitters and maturational stages of individual neurons Sarnat [2, 32].

Neuropathological Changes in Brain Tissue in Cerebral Palsy

Because the aetiologies and mechanisms of pathogenesis of cerebral palsy are so numerous and varied, there are no precise neuropathological findings that are definitively diagnostic of cerebral palsy but rather many findings are consistent with this clinical condition and indeed are highly likely to be associated. The findings are related to the cause of the lesions: brain malformations of genetic or epigenetic cause during embryonic and foetal life; hypoxic-ischaemic encephalopathy; toxic encephalopathies such as bilirubin encephalopathy, heavy metal intoxications and foetal alcohol spectrum disorder; congenital infections; traumatic brain injury at birth or after; metabolic encephalopathies due to inborn errors of metabolism; genetic mutations and syndromes and chromosomopathies. Each of these categories will be considered below.

Amongst recent discoveries of relevance is the expression of the extracellular matrix proteoglycan *keratan sulphate*. This molecule is secreted by glial cells and in the foetus is in highest concentration in the thalamus and globus pallidus, but it also is expressed in the cortex, particularly in the molecular zone and deep layers [34]. It binds to neuronal membranes, except for terminal dendrites and dendritic spines, and repels glutamatergic (excitatory) axons while facilitating GABAergic (inhibitory) axons and explains why axosomatic synapses are inhibitory and axodendritic synapses are excitatory. Furthermore, keratan sulphate envelops axonal fascicles, such as those of the corticospinal tract, to prevent axons from exiting the fascicle before reaching their programmed destination and to prevent unrelated axons from grey matter structures *en route* from entering the fascicles [34] (Fig. 34.1). Keratan sulphate deposition in the internal capsule begins as early as 12 weeks gestation and continues throughout foetal life, long before myelination is initiated at 3 months postnatally in term infants.

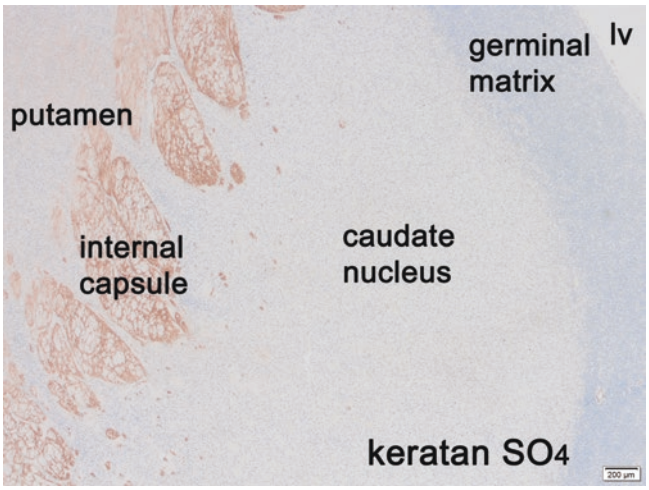

Fig. 34.1 Coronal section of brain of 20-week gestation foetus at level of the corpus striatum (caudate nucleus and putamen). Keratan sulphate (KS) immunoreactivity envelops axonal fascicles of the anterior limb of the internal capsule to insulate them and prevent axonal exiting too early. It may be impaired with loss of KS in some conditions that lead to cerebral palsy, such as hypoxic-ischaemic encephalopathy. The germinal matrix of pre-migratory neuroblasts and glioblasts contains no KS. *lv* lateral ventricle

Though alterations in the cerebral cortex in cerebral palsy have been described for a century or more only recently has the U-fibre layer just beneath the cortex been recognized as altered in various pathological states. U-fibres are short association axons arising from layer 6 of the cortex that interconnect other parts of the same gyrus and the immediately adjacent gyri. In the U-fibres beneath focal cortical dysplasias, there often is an excessive number of heterotopic neurons that form abnormal synaptic plexi that contribute to epileptic networks [35]. Whether U-fibre abnormalities are present in or contribute to cerebral palsy as they do to epilepsy remains unknown.

Cerebral Dysgeneses or Malformations Associated with Cerebral Palsy

With the advent of improved neuroimaging techniques, brain malformation are now demonstrated to be a much more major cause of neurological dysfunction than previously suspected. All involve interference with normal developmental

processes and architecture of the various parts of the brain, and also involve maturation of individual neurons and glial cells. These are all processes related to *timing*, the key factor in pathogenesis [1, 2]. The following is a list of the most frequent genetically determined brain malformations that can cause cerebral palsy and also often are the underlying basis of epilepsy.

Tuberous Sclerosis Complex (TSC), Hemimegalencephaly (HME) and Focal Cortical Dysplasia Type II (FCD II): Disorders of the mTOR Signalling Pathway

TSC is a common genetic disease that is complex genetically because it is both a germline mutation transmitted as an autosomal dominant trait and also a post-zygotic somatic mutation resulting is disturbance of the mTOR signalling pathway. Dysplastic megalocytic neurones and glial cells are characteristic in the cortical tubers, but neurons are absent from the periventricular nodules and subventricular giant cell astrocytomas. Activation of the mTOR pathway also occurs in HME and in FCD II and the histopathological findings are similar [36, 37]. Inflammation in the foetal brain in cortical tubers of TSC is another factor that may contribute to epilepsy and other deficits including cognition and motor impairment [38].

Holoprosencephaly (HPE)

Some 14 genes are now implicated in the aetiology of this midline cerebral hypoplasia/aplasia, which still account for only about 25% of total cases studied, hence many more genetic deletions or mutations are yet to be discovered. Nevertheless, the phenotype is very similar in all; hence, this malformation is an example of poor genotype:phenotype correlation. Clinical expression is global developmental delay and intellectual deficit, anosmia (due to arrhinencephaly), midfacial hypoplasia, often epilepsy and spastic diplegia. Neuropathological findings are extensive and may involve posterior fossa structures as well as non-cleavage of the cerebral hemispheres (persistent prosencephalon) and thalami, agenesis of forebrain commissures and a medio-lateral gradient of cerebral cortical dyslamination with precocious synaptogenesis but in an abnormal patchy cortical distribution, 3rd venticular and aqueductal atresia leading to foetal hydrocephalus and retinal and ocular malformations [33, 39–41]. Excessive keratan sulphate envelops not only axonal fascicles but also insulates individual axons within fascicles, that may partially explain why up to 40% of children with this malformation do not have epilepsy despite extensive cortical dyslamination and architectural disorganization [42].

Septo-optic-pituitary Dysplasia (du Morsier syndrome)

This disorder overlaps with HPE because both involve midline structures of the brain, but they are not the same and have different genetic bases. The three major components, for which any two are sufficient for diagnosis, are absence of the septum pellucidum, hypoplasia of the optic nerves and chiasm, and anterior pituitary insufficiency. A fourth component in most cases is hypoplasia or even agenesis of the olfactory bulbs and tracts. The corpus callosum and anterior commissure usually are well formed, by contrast with other 'midline defects'. Pituitary insufficiency ranges from isolated growth hormone deficiency to panhypopituitarism but is a treatable aspect with hormonal replacement therapy. One of the most disabling components is visual impairment. Intellectual deficit and motor deficits as cerebral palsy are inconstant features but occur in a significant number of cases. Epilepsy occurs in a minority of patients. Both epilepsy and cerebral palsy (spastic hemiplegia or diplegia) are greatly increased in prevalence if the septo-optic-pituitary dysplasia is accompanied by unilateral or bilateral schizencephaly, as it is in at least a third of cases.

Schizencephaly

This condition is a unilateral or bilateral cerebral hemispheric cleft, usually in the position of the Sylvian fissure, that extends to the ventricular wall where it is forms a thin 'pial-ependymal membrane' with no intervening grey or white matter, or is lined entirely by dysplastic grey matter including at the ventricular surface with no underlying white matter. Developmentally it often is associated with defective telencephalic flexure that forms the operculum and later the lateral cerebral (Sylvian) fissure [43], but various genetic mutations have been associated as well. Since all three lips are derived from the ventral surface of the primitive telencephalon after cleavage of the embryonic prosencephalon, genes acting in a ventro-dorsal gradient in the vertical axis of the neural tube are most implicated [43]. The three 'lips' of the Sylvian fissure (frontal, temporal and insular) nearly always exhibit polymicrogyria (see below) and disorganized cortex that often is epileptogenic. Unilateral schizencephaly is usually associated with contralateral spastic hemiparesis and bilateral schizencephaly with diparesis. Septo-optic-pituitary dysplasia is a frequent associated malformation (see above).

Numerous genetic mutations and chromosomopathies have been demonstrated in schizencephaly, and familial cases also are described, but the known genetic mutations remain the minority of those patients studied [44–46].

Polymicrogyria (PMG)

Gross appearance in MRI and macroscopic neuropathological inspection exhibits clusters of excessively small gyri separated by shallow sulci. Many of these microgyri are actually fused through discontinuities in the pia mater at their lateral surface so that the molecular zone (layer 1 of the normal 6-layered neocortex) becomes continuous between adjacent gyri at sites of pial discontinuity [44, 47–49]. The normally cell-sparse molecular zone is filled by axodendritic synapses from deeper layers of the cortex. Pial discontinuities or gaps enable synaptic short-circuits to form between the molecular zones of adjacent fused gyri and these abnormal circuits may become epileptogenic. In some cases, lamination of the other layers of cortex in those regions may be indistinct and neurons disoriented or displaced. In 'unlayered' PMG, the cortex is thinned with only a molecular layer and a neuronal layer without distinct laminar organization. Even in layered forms, only four distinct layers of the cortex may be seen, corresponding to a molecular layer and two layers of neurons separated by an intermediate layer of a few cells and myelinated fibres [50]. In other cases, all 6 layers of cortex are still recognized. PMG may occur in any part of the cerebral cortex, but is most frequent in the perisylvian region involving all three 'lips' of the lateral cerebral fissure and, at times, lines the deep cleft extending to the ventricular wall to become schizencephaly (see above). Polymicrogyria should be distinguished from ulegyria, an acquired ischaemic insult in foetal life (see *Cerebral Ischaemia…* below).

One of the most important anatomical features of polymicrogyria is that the microgyria frequently are fused on their lateral surfaces because of discontinuities of the pia mater, so that the molecular zones of adjacent microgyria are continuous [44, 47]. These gaps also enable synaptic short-circuitry between adjacent gyri and the molecular zone contains excitatory glutamatergic axodendritic synapses, so that the ratio of excitation to inhibition is shifted in favour of excitation and epileptogenesis [51].

Apart from its potential to cause focal epilepsy and severe neonatal seizures [52], PMG may impede normal motor function arising from the involved hemisphere, especially if in the frontal lobe or perisylvian region. This impaired motor function is expressed clinically as a contralateral spastic hemiparesis if PMG is unilateral or diparesis if bilateral, involving both gross and fine motor functions. Cognitive skills also are impaired, especially if perisylvian polymicrogyria is bilateral [53].

Lissencephaly Types I and II

All lissencephalies are primary disorders not only of neuroblast migration but of organization of the cortical plate, and spastic diplegia is one of the prominent clinical manifestations, along with intellectual/cognitive impairment and epilepsy. In lissencephaly II, particularly Walker–Warburg syndrome, the malformation is not limited to the forebrain but also involves the cerebellum, brainstem and spinal cord.

Periventricular Nodular Heterotopia, Subcortical Laminar ('band') Heterotopia and Scattered Deep White Matter Heterotopia

This genetic disorder (several mutated genes now identified) of neuroblast migration often results in microcephaly and in spastic diplegia, in addition to intellectual/cognitive impairment and epilepsy.

Cerebral Ischaemia and Infarcts in the Foetal and Perinatal Periods, Including Porencephaly

Infarcts of the foetal brain may occur at mid-gestation or late in gestation, depending upon the cause. They may be ischaemic or haemorrhagic in nature, but either often results in spastic hemiplegia or diplegia postnatally. Ischaemic infarcts are often due to transitory systemic hypotension because of some event in the materno-placental-foetal circulation, such as an episode of cardiovascular shock in the mother. The distribution of such infarcts often is in the watershed zone in the overlapping distributions of the three major cerebral arteries and/or in the tegmental watershed zone of the brainstem which also can result in Möbius syndrome, dysphagia and respiratory insufficiency in the postnatal period [54]. With re-perfusion, cortical and subcortical infarcts may change in character from 'white' ischaemic lesions to 'red' haemorrhagic lesions because of damage to endothelial cells of fragile capillaries that rupture when normal blood pressure is restored. Scattered cortical and subcortical white matter micro-infarcts also can occur.

In preterm foetuses or neonates of 35 weeks gestation or less, germinal matrix haemorrhages in the subventricular zone are frequent occurrences because of immaturity of thin-walled vessels and sudden changes in vascular dynamics, particularly on the venous side. Such germinal matrix haemorrhages may be small and confined to the pre-migratory cells of the periventricular region, in which case they result in little or no neurological deficit later, or the haemorrhages may rupture into the ventricle or extend in the other direction into the cerebral parenchyma where they nearly always cause deficits, often motor in nature as cerebral palsy. In autopsies of adults who have sustained intracerebral haemorrhages in adult life as a form of 'stroke', evidence of haemosiderin deposit in the brain tissue persists even many years or decades later; in foetuses and neonates, by contrast, intrace-

rebral haemorrhages do not leave permanent haemosiderin deposits because they are carried away by macrophages [55].

Ischaemic lesions of the cerebral cortex do not always lead to frank infarction in a defined anatomical zone but may cause selective loss of neurones from specific layers, known as *laminar necrosis*.

Ulegyria

Chronic prenatal ischaemia in late gestation after convolutions is formed that may cause cellular loss and atrophy of the walls of gyri with relative better preservation of gyral crowns that cause mushroom-shaped small gyri known as *ulegyria*. The narrow 'stalk' of the gyrus is cortex almost totally depleted of neurons and arterioles of the overlying leptomeninges often exhibit fibrotic subintima and calcification in their walls [56, 57]. Compression of vessels in the depths of sulci by either mechanical distortion or oedema was the explanation of increased vulnerability of the gyral wall [57]. Ulegyria occurs less frequently now than 50–100 years ago. It must be distinguished from polymicrogyria, more difficult by MRI than by histopathological examination.

Porencephaly

Porencephaly is a large cyst usually in the distribution of the middle cerebral artery on one side due to occlusion of that vessel often at mid-gestation. The resulting cyst is the end-result of the necrotic infarcted brain tissue having been removed by macrophages. The margins of the cyst may show focal cortical dysplasia type Ia because this zone was ischaemic but did not become infarcted and the ischaemia caused a maturational arrest of cortical lamination [58]. The reason for a middle cerebral artery to become occluded at mid-gestation is an incompletely resolved issue, but it may be due to segmental lack of the *elastica interna* at the junction where the middle cerebral artery attaches to the internal carotid artery, with subsequent narrowing and occlusion of the lumen (Sarnat HB, unpublished). Porencephaly is almost always associated with spastic hemiplegia.

Brainstem Watershed Infarcts

A 'watershed' is a term introduced into geology and agriculture as a narrow strip of land between two rivers or streams that receives some water supply from both sources. But this supply is the terminal irrigation of both, so that blockage of one enables the strip to survive because of its alternative supply but the median strip perishes in times of drought in which the water level of both streams is low and irrigation poor with the terminal part suffering first [54]. Extrapolated to the arterial supply of the human brain, transitory systemic hypotension in the mother or the foetus may result in infarction of 'watershed' territories between major cerebral vessels. This is best known as watershed infarction between the territories of the anterior and middle cerebral arteries or the middle and posterior cerebral arteries.

The brainstem is supplied by a single midline basilar artery, though this vessel results from fusion of the transitory paired longitudinal neural arteries of the rhombencephalic region of the embryonic neural tube [54]. Hypoperfusion of the vertebra-basilar circulation in foetal life, even if only transitory, can result in symmetrical infarcts of the tegmentum of the midbrain, pons and medulla oblongata [54, 59, 60]. The structures affected include the abducens nucleus and the intramedullary loop of the facial nerve around it to produce Möbius syndrome; more extensive lesions may involve the hypoglossal nucleus with atrophy and fasciculations of the tongue, the nucleus ambiguus to produce congenital dysphagia and the nucleus solitarius that results in central hypoventilation or 'lack of respiratory drive' [54, 61]. The ventrally situated cortico-spinal/ pyramidal tract is spared, however, so that spastic diparesis may be mild or not evident at all. If present, pyramidal tract signs originate from supratentorial involvement of the internal capsule or motor cortex.

Periventricular Leukomalacia, White Matter Gliosis, and the Roles of Iron and Zinc

Periventricular leukomalacia (PVL) is a lesion of mainly the late second and early third trimesters of gestation and remains a frequent lesion of preterm infants but occurs only rarely after 35 weeks gestation and is a major cause of spastic diplegia in premature infants who survive other life-threatening challenges in the neonatal period.

Historical Perspective and Descriptive Morphology

The English physician WJ Little [62] was the first to describe cerebral palsy, in the mid-nineteenth century, and further astutely observed that the majority of affected children had been born prematurely or had suffered asphyxia at birth. Virchow, the *father of pathology*, provided the first neuropathological description of brain lesions at autopsy of children with cerebral palsy, and at the same time Parrot [63] described white matter infiltrates of focal foamy macrophages, infarction and sometimes haemorrhage in the periventricular regions (PVL). The hypothesis of venous stasis

as the primary pathogenesis of PVL was first proposed by Schwartz [64] and reiterated years later [65], but is more related to germinal matrix haemorrhages.

The typical histopathological findings in brain thus have been known for a century and a half. In the mid-twentieth century Banker and Larroche [66] recognized the association of perinatal hypoxic/ischaemic encephalopathy with later cerebral palsy; they further identified white matter lesions (PVL) as the principal neuropathological substrate of spastic diplegia. Classical systematic descriptions by Margaret Norman of the Bristol, UK were published in Greenfield's Neuropathology in the second edition (1963). Other mid-twentieth century perinatal neuropathologists who contributed detailed morphological descriptions of the lesions of both grey and white matter lesions in preterm and term neonates in composite works over decades later published as textbooks, include those of Towbin [67], Friede [68], and Courville [69]. Gilles et al. [70] emphasized the hypertrophic and damaged astrocytes around focal necrotic lesions in white matter. The observations by these pioneers of perinatal neuropathology are timeless and as valid today as they were when they first appeared, similar to the meticulous descriptions of developmental neuroanatomy by Ramón and Cajal in the late nineteenth and early twentieth centuries that also have stood the test of time. More recently, I also described the details of microscopic histopathological findings [55] and an excellent current neuropathological description of the white matter lesions was provided by Haynes and Folkerth [71].

With the advent of immunocytochemistry in the past few decades, the application of more recent neuropathological techniques has not only extended the histopathological findings but has further validated them. Glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) immunoreactivities confirmed that the majority of reactive proliferative glial cells in zones of infarction and myelin breakdown indeed were astrocytes [72]. Cellular markers of oxidative stress are observed in white matter foci [71] and heat-shock proteins such as α-B-crystallin, also upregulated at epileptic foci [73] and inflammatory markers also usually are evident if sought with appropriate immunocytochemical markers (Sarnat HB, personal observations).

The twenty-first century has seen a change in relative frequency of the white matter lesions in perinatal hypoxic/ischaemic encephalopathy, in large part thanks to improved foetal and neonatal care and the ability to detect early lesions prenatally by ultrasound and foetal MRI. Diffuse white matter injury, consisting of diffuse gliosis, incorporates PVL which is now defined neuropathologically as focal white matter necrosis in addition to the almost universal gliosis [71]. Grey matter lesions also nearly always occur as well and are not limited to the cerebral cortex (see below), but the most evident and constant lesions still occur in the immature and yet unmyelinated or early myelinating white matter. The large foci of necrosis with cystic changes which were so prominent in late nineteenth and early to mid-twentieth century descriptions are less frequent or not as prominent, and the fatty component secondary to myelin degeneration also is not as striking in microscopic sections. Rather, multiple smaller or microscopic foci of necrosis are more frequently observed, associated with diffuse reactive gliosis [71]. Some preterm neonates develop spastic diplegia and have many of the risk factors for PVL, yet white matter lesions are not seen by MRI pre- or postnatally; post-mortem microscopic neuropathological lesions often can be demonstrated, however [74, 75].

Impediment of Oligodendrocytic Lineage in PVL

Impaired myelination in lesions of PVL is well documented at autopsy for many years, though myelination of the centrum semiovale (deep subcortical white matter) is not normally initiated until about 3–4 months of age post-term by MRI or a month earlier by neuropathological criteria using the Klüver and Barrera [18] method of luxol fast blue myelin stain [19–22] and confirmed more recently with myelin basic protein immunoreactivity. The pre-oligodendrocyte is the principal cellular target in white matter injuries of preterm neonates and in animal models [75–78]. Pre-oligodendrocytes are generated from progenitor cells and are the principal phase of oligodendrocytic lineage during late gestation and the premature postnatal period. Pre-oligodendrocytes can be induced to form in cell culture from undifferentiated progenitor cells [79]. Arrested pre-oligodendrocyte maturation explains failure of myelination in preterm infants [80]. At 15–20 weeks gestation thalamic projections to the sub-plate zone occur and at 27 weeks subplate neurones extend their axones to more superficial parts of the cortex [81, 82]. During these periods, axonal growth cones and immature axons that have reached their target are particularly vulnerable to ischaemic injury. Zinc is an essential metal for transcription factors of several genes essential to pre-oligodendrocyte cells (see below). Oligodendrocytic precursors and lineages and genetic mutations are recently demonstrated in murine and stem cell models [79, 83], but human data are still sparse. Oligodendrocyte heterogeneity is now demonstrated in adults with multiple sclerosis [84], but data from children with cerebral palsy are few. Olig2 is a good antibody available as a selective tissue marker of oligodendrocytes.

Astrocytic Role

The white matter lesions in PVL usually show some degree of gliosis or astrocytic proliferation (astrocytosis). Though traditionally regarded as a CNS 'scar tissue', astrocytes

probably play a much more important role than simply a reactive change after the fact. In vanishing white matter disease, a progressive leukodystrophy unrelated to PVL, the principal target is deep subcortical white matter (but sparing of U-fibres) and the pathogenesis is likely astrocytic dysfunction and disconnection with their relation to microglia (resident macrophages of the CNS) and to neurones [85]. Microglial activation also is seen in PVL.

Iron

Iron is the most abundant metallic ion in the foetal brain, but not entirely due to intravascular haemoglobin in erythrocytes. Iron plays an important role in neurotransmitter metabolism [26] and in oligodendrocyte function in myelin production [86–89]. Foetuses and preterm neonates with iron-deficiency anaemia have delayed motor and cognitive development [90, 91]. They later show prolonged latencies but with normal amplitudes of visual and auditory evoked potentials, likely due to delayed myelination [92–94]. Dietary iron supplementation therefore is an important restorative therapeutic intervention not only for anaemia but for cerebral maturation as well [75].

Zinc

Zinc is the second most abundant metallic ion in the foetal brain, in large part because of 'zinc-finger' genes of development in which zinc ions are structurally incorporated into transcription factors. Some of these transcription products cooperate with Olig2 to promote oligodendrocytic differentiation in precursor cells [95]. Oligodendrocyte precursors are generated in the same ventral part of the neuroepithelium of the primordial spinal cord as are motor neurones, and *Sonic hedgehog* (*SHH*) signalling induces not only motor neurone differentiation and maturation but also stimulates oligodendrocyte precursors [96, 97]. Gli3 zinc finger transcription factor is a transducer of *SHH* signalling that also induces oligodendrogenesis [98]. NG2-glia in both immature and mature brain also rely upon zinc-finger and homeodomain proteins for precursor cells [99]. Zinc-dependent endopeptidases, by contrast, enhance neuronal differentiation and suppress oligodendrocytic precursors in hippocampus [100]. Zinc-containing axonal mossy fibres are prominent in hippocampus and olfactory bulb. Zinc deficiency or metabolic blockage in zinc metabolism are, therefore, a basis for altered maturation of oligodendrocytes that later is expressed as delayed or deficient myelination, also a factor in the pathogenesis of periventricular leukomalacia. Two types of oligodendrocyte precursors lead to myelinating cells or non-myelinating oligodendrocytes that ensheath unmyelinated axones which are abundant in the central nervous system as well as in autonomic, pain-transmitting and some other somatosensory peripheral nerves.

Grey Matter Lesions in PVL

Though the most prominent neuropathological lesions by both neuroimaging and neuropathology occur in the subcortical white matter, grey matter abnormalities also are found [101]. Indeed, some of the white matter injury involves axonopathies of fibres emanating from damaged cortical pyramidal neurones of layer 5. Not only cortical neurones, but those of the thalamus exhibit accelerated apoptosis with neuronal loss, axonal degeneration, gliosis and general atrophic changes [102, 103]. Similar thalamic changes are seen in experimental neonatal hypoxia in the rat [104]. Within the cortex, laminar necrosis is seen in severe human cases [105].

Basal Ganglia

The corpus striatum (caudate nucleus and putamen) and especially the globus pallidus are affected in perinatal hypoxic/ischaemic encephalopathy and particularly in spasticity with dyskinesias. In addition to neuronal loss and gliosis, there is hypermyelination of axons, particularly found at autopsy in older infants and children who had suffered perinatal asphyxia. The gross cut section has the appearance of a marbled surface, hence the terms *status marmoratus* or *état marbré*. On microscopic examination, axoplasm of such fibres is thickened but without degeneration and is surrounded by a thicker than normal myelin sheath. Regeneration has been suggested. It is important to recognize for dyskinetic forms of cerebral palsy in which the basal ganglia are implicated, that there are no direct pallidospinal or striatospinal (caudatospinal or putamenospinal) tracts in the human brain, so that the deep telencephalic nuclei influence muscular control in the spinal cord via ascending fibre projections that influence cerebral cortical function and through the corticospinal tract as the final descending pathway.

Cerebellum

The cerebellar cortex in perinatal hypoxic/ischaemic often has atrophic folia that microscopically exhibit neuronal loss of Purkinje cells more than granule cells. The dentate nucleus is relatively spared except in the most severe cases. There are no direct cerebellospinal tracts, so cerebellar influence on motor function is mediated by ascending dentatothalamic (to the ipsilateral ventrolateral thalamic nucleus) and then thalamocortical projections with the corticospinal tract being the

final descending pathway. Olivospinal and rubrospinal pathways of the cerebellar system do exist, but these bulbospinal tracts from the brainstem are very small in humans and their function is not well defined.

Congenital Infections

In cytomegalovirus disease (CMV), viral invasion of endothelial cells leads to multiple cerebral infarcts of various sizes ranging from micro-infarcts to porencephaly and multicystic encephalomalacia [106]. This pathogenesis may be a factor, but to a lesser extent than in CMV, in other congenital infections including Herpes, Zika and toxoplasmosis. Spastic diplegia is a frequent sequel of congenital infections, along with other neurological handicaps including cognitive, behavioural and epilepsy.

Inborn Metabolic Encephalopathies

Mitochondrial cytopathies that present in foetal life or early infancy, such as Leigh encephalopathy, often cause periventricular necrosis. Excessive apoptosis of neuroepithelial cells and neuroblasts is one reason, but mitochondrial dysfunction in immature brain involves endothelial cells of capillaries earlier and more severely than neurones and glial cells. This difference from adult-onset mitochondrial encephalomyopathies has been demonstrated in ultrastructural studies of muscle biopsies in neonates, infants and toddlers with genetically confirmed mitochondrial diseases, termed 'mitochondrial angiopathy' [107]. Hypermetabolic neurones with excessive mitochondrial activity are demonstrated at epileptic foci in infants and children [108].

In many non-mitochondrial inborn errors of metabolism, including aminoacidurias and organic acidurias, affected children have motor deficits that may be progressive. Widespread structural abnormalities in cortical lamination are demonstrated in some, such as methylmalonic aciduria [31].

Ventriculomegaly and Hydrocephalus

Ventriculomegaly is enlargement of the lateral ventricles or the entire ventricular system. It is the most frequent brain abnormality detected by foetal ultrasound. It may be secondary to white matter volume loss, as colpocephaly (selective enlargement of the occipital horns) associated with total agenesis of the corpus callosum or generalized cerebral atrophy due to chronic foetal ischaemia.

Hydrocephalus is ventriculomegaly with increased intraventricular pressure, usually obstructive from aqueductal atresia, a lesion at the level of the foramen of Munro (that produces unilateral or asymmetrical lateral ventriculomegaly) or an obstructive lesion of the posterior fossa or fourth ventricular outlet such as a Dandy–Walker malformation. Congenital hydrocephalus causes spastic diplegia from stretching of corticospinal fibres, but early relief of intraventricular hypertension may minimize the spastic diplegia. A characteristic finding in neonatal hydrocephalus is a shrill, high-pitched cry. This is the result of corticobulbar tract impairment with pseudobulbar palsy; the vocal cords develop increased tone (spasticity) and lie closer together than normal, producing a higher than expected pitch during crying. Pseudobulbar palsy also may cause dysphagia with aspiration (nucleus ambiguus), can impair central respiratory rhythms (nucleus/tractus solitarius; pre-Bötzinger nucleus), and produce bradycardias and increased intestinal peristalsis with diarrhoea (dorsal motor vagal nucleus).

Microcephaly

Microcephaly indicates a small head with a circumference more than 2 SD below the mean for conceptional (corrected) age, as measured clinically with a tape measure or by prenatal ultrasound. The cause of microcephaly is usually *micro-encephaly*, a small brain that does not stimulate the cranial vault to grow further. Microcephaly is especially evident, though not required to define, if the other standard growth parameters such as weight and length are within the normal range. Most, but not all, children with primary genetic microcephaly have spastic diparesis; neuropathological findings in the brain include decreased volume of both grey and white matter, pachygyria and abnormalities of cortical lamination and fewer than normal but not dysplastic neurons. If microcephaly is secondary to brain damage such as multicystic encephalomalacia, spastic diplegia is almost universal. Most patients with primary microcephaly do not have epilepsy, but one genetic form has severe global developmental delay and seizures but with variable motor impairment [109].

Rarely, microcephaly may be secondary to craniosynostosis, in which case the brain is normal unless the growing brain is restricted and compressed by failure of the cranium to grow if multiple sutures are not opened (e.g. trigonocephaly) and atrophy ensues. But single or paired suture fusion, such as of the sagittal suture alone or the coronal sutures alone, may cause an abnormal head shape (scaphocephaly; brachycephaly) but this shape compensates for intracranial volumetric space and neurological dysfunction including cerebral palsy may be spared.

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Multiple Choice Questions

1. In dyskinetic cerebral palsy, the basal ganglia are implicated. These deep telencephalic nuclei mediate their influence on the spinal cord via the:
   (a) Pallidospinal tract
   (b) Striatospinal tract
   (c) Corticospinal tract
   (d) Small multiple bulbospinal tracts
   (e) All of the above
   Correct answer: (c)
2. Within the spectrum of neuropathological findings in the brains of infants and children with cerebral palsy, the most frequent is:
   (a) Extensive loss of upper motor neurons from layer 5 of the cerebral cortex
   (b) Loss of granular neurons from layers 2 and 4 of the cerebral cortex
   (c) Extensive gliosis and inflammatory cell infiltration of the neuropil
   (d) Subcortical white matter gliosis and axonal loss (periventricular leukomalacia)
   (e) Loss of motor neurons in the ventral horns of the spinal cord
   Correct answer: (d)
3. Which of the following are not a cause of cerebral palsy?
   (a) Infantile spinal muscular atrophy
   (b) Focal cortical dysplasia type I
   (c) Hereditary sensory-motor neuropathy
   (d) Krabbe disease (galactosyl-ceramide-beta-galactosidase deficiency)
   (e) All of the above
   Correct answer: (e)
4. Cerebral palsy in cerebral malformations:
   (a) Correlates with the degree of histological disorganization of the cerebral cortex
   (b) Correlates with risk of epilepsy
   (c) Correlates with degree of gliosis
   (d) Correlates with associated inflammatory cells
   (e) None of the above.
   Correct answer: (e)

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Genetics and Genomics of Cerebral Palsy

35

Jan Friedman and Clara van Karnebeek

Learning Objectives

To understand/gain insight into:

- The type of genetic aberrations underlying cerebral palsy and of the mode(s) of inheritance.
- The reported yield of genetic investigations (including chromosomal micro-array analysis, exome sequencing) in cerebral palsy.
- The difference in yield of genetic testing between typical and atypical cerebral palsy patients.
- The importance of establishing an underlying diagnosis in patients with cerebral palsy.

Highlights

- At least 4% of patients with cerebral palsy have disease-causing copy number variants, and at least 14% have disease-causing single nucleotide variants or indels.
- In patients in whom cerebral palsy-like neuromotor dysfunction occurs with additional malformations or neurodevelopmental abnormalities, the rate of disease-causing genomic lesions is more than twice as high.
- Aberrations of many different genetic loci can produce a cerebral palsy-like phenotype.
- Most, but not all, gene or chromosomal mutations that cause cerebral palsy occur de novo.
- Recognizing the cause of cerebral palsy in an affected patient is essential to providing optimal clinical management, including precision therapy.
- Genome-wide (exome or genome) sequencing is indicated in the initial work-up of patients with cerebral palsy, especially those who have additional neurodevelopmental abnormalities or malformations.

Introduction

Cerebral palsy (CP) is not a homogeneous disease entity but rather an etiologically diverse group of conditions characterized by abnormal movement or posture with onset early in development [1–3]. It has been known for more than 50 years that some patients with clinical features of cerebral palsy have a genetic syndrome or inherited metabolic disorder [4, 5], but for a long time such cases were considered to be highly exceptional. We now know that they are not — it has become apparent in the past decade that many patients with developmental abnormalities of motor function have an underlying genetic disease of major effect, such as a Mendelian disorder or chromosomal abnormality.

The structural and/or functional central nervous system abnormalities that underlie CP may have their origin at conception, during embryonic or foetal development, during the perinatal period, or in early childhood. A major genetic cause is most likely when the condition has obvious prenatal onset, but the clinical features of CP may not become manifest until later in life in other instances. Non-genetic factors, such as teratogenic exposures, hypoxia, hemorrhage or infections, may also cause CP, and in some other patients the cause is a

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combination of non-genetic and genetic factors. 'Genetics' is definitely plural when referring to CP.

Patients with CP are often classified clinically into spastic, hypotonic, dystonic (also called 'dyskinetic'), ataxic, and mixed subgroups and by the limbs involved (diplegia, hemiplegia or quadriplegia, and occasionally other patterns) [1–5]. Each of these clinical subgroups and patterns of involvement is also etiologically and genetically heterogeneous, and while certain major genetic forms of CP characteristically produce only one particular kind of involvement, the clinical presentation of other genetic forms of CP is variable [1, 6].

Clinical definitions of CP require that the condition be non-progressive, and developmental abnormalities of movement or posture that become worse with time are sometimes called 'atypical CP' or 'cerebral palsy mimics' [6, 7]. Distinguishing progressive from non-progressive neuromotor abnormalities is important for clinical management but may present difficulties in genetic analysis of these conditions for several reasons [8]. Firstly, disease progression occurs over time and may not be apparent when a child is initially evaluated. Secondly, the rate of progression may be very slow, and the functional loss may not become apparent until later in life. Thirdly, some patients are very severely involved from birth, and it may not be possible to recognize disease progression clinically. Fourthly, disease progression may not affect motor function but become apparent in other ways, such as intractability of seizures to treatment, loss of vision or speech, or cognitive decline. In addition, genetic diseases that can cause CP are often quite variable in their manifestations and course from patient to patient, so that disease progression may be obvious in some individuals but not in others with the same condition. Finally, specific treatment is available for some diseases that may present as CP [9], and the treatment may prevent progression of the neuromotor symptoms.

In this chapter, we consider the genetics and genomics of both non-progressive and progressive neurodevelopmental movement disorders because almost all reported studies include some patients who have typical CP and others who are atypical or may become so later in their course. The information is organized by study design: twin and other family studies (without genetic testing), association studies, studies of chromosomal abnormalities or genomic copy number variants, studies of Mendelian diseases caused by single nucleotide variants or indels, and epigenetic studies. This organization also generally reflects the time when the studies were done, with genome-wide sequencing and epigenetic studies being most recent, and the others, earlier.

Twin and Other Family Studies

Hundreds of studies have been published that include twins with CP, but such studies are difficult to interpret with respect to genetic causation because being born of a twin pregnancy is itself strongly associated with the occurrence of CP. Luu and Vohr [10] and Pharoah and Dundar [11] summarized data from CP registry studies and found a substantially greater frequency of CP in twins than in birth registries for the same jurisdictions. CP was reported in 6.3–12.6 per 1000 twins who survived infancy in comparison to 1.0–2.3 per 1000 surviving singleton infants. A population-based study performed through the Medical Birth Registry of Norway found 3649 children who developed CP and 22,558 pairs of twins among 2,036,741 infants born between 1967 and 2002. [12] The prevalence of CP was three times greater among the twins (5.1 per 1000) than among singleton births (1.7 per 1000). After reviewing such data, Briana and Malamitsi-Puchner [13] emphasized the importance of low birth weight and premature delivery, which frequently occur in twin pregnancies, in mediating the development of CP.

Twin studies have been used for almost 150 years to infer genetic causation of familial traits based on recognition that monozygotic twins share all of their genes in common, while dizygotic twins resemble ordinary sibs, sharing about half of their genes [14]. The studies discussed in the previous paragraph comparing the rate of CP in twins to that in singleton pregnancies or the general population ignore zygosity and thus cannot be used to assess the importance of genetic factors in the occurrence of CP.

A study of the population-based Western Australia CP Registry identified 74 sets of twins born between 1956 and 1985 in which one or both members of the pair had CP. [15] The rate of concordance for CP in monozygotic twins was significantly higher than that in dizygotic twins ($p = 0.0026$). In contrast, concordance for CP was observed in 4 (20%) of 20 monozygotic twin pairs and 10 (40%) of 25 dizygotic twin pairs in a series collected by a single physician over a 21-year period. [16] The fact that concordance was not complete among monozygotic twins is consistent with the known etiological heterogeneity of CP and formally proves that all cases are *not* caused by major genetic factors.

Very few studies of CP in twins have confirmed zygosity by genetic testing, but monochorionic placentation is strongly associated with the occurrence of CP in twins. [17, 18] Almost all dizygotic pregnancies have dichorionic placentation, and most monozygotic pregnancies are monochorionic, but about 30% of monozygotic pregnancies are dichorionic [19]. Chorionicity is, therefore, an imprecise surrogate for zygosity. The proportion of twin pregnancies that is monozygotic, rather than dizygotic, varies greatly in different populations and has changed in the last few decades as a result of fertility treatments that increase the frequency of pregnancies with two or more genetically distinct foetuses.

The association of monochorionic placentation with CP may largely be attributable to the occurrence of placental vascular anastomoses between the circulatory systems of the twins [20, 21]. Monochorionic placentation is also associated with increased frequencies of intrauterine death of one

or both twins, preterm delivery, severe discordance in birth weight between the twins, foetal growth restriction, and congenital anomalies, all of which are also associated with the occurrence of CP [10, 11, 13].

No twin studies have been reported that assess the effect of genetic factors on the occurrence of CP in proven monozygotic versus dizygotic (or monochorionic vs. dichorionic) twins after removing the effects of placental vascular anastomosis, intrauterine death of one twin, preterm delivery, severe birth weight discordance, foetal growth restriction and other congenital anomalies.

Studies that have compared the frequency of CP in co-twins of unknown zygosity to the frequency of CP in sibs of children with CP born of singleton pregnancies have found higher rates of co-occurrence of CP in the twin sibs.

A Norwegian population-based record linkage study [12] found the prevalence of CP to be 79/1000 in the co-twins of children with CP, 15/1000 in the sibs of singleton children with CP, 8.5/1000 in the children of parents with CP, 2.6/1000 in the second-degree relatives of children with CP, and 2.5/1000 in the third degree relatives of children with CP. The prevalence of CP was 1.5/1000, 1.6/1000 and 1.6/1000, respectively in first-, second- and third-degree relatives of individuals *without* CP in this study. A subsequent publication expanded this investigation by adding four more years of data to include a total of 5707 children with CP and 26,485 twin pairs among 2,297,408 children who survived the neonatal period [22]. In the expanded study, the co-twins of children with CP had a 27-fold greater than expected risk of having CP, and the full sibs of children with CP born of singleton pregnancies had a 6.4-fold greater risk of having CP. The sibs of children with CP born of singleton pregnancies also had higher than expected risks of stillbirth, neonatal death, intellectual disability, autism spectrum disorder, deafness, blindness, epilepsy, attention deficit hyperactivity disorder and schizophrenia. All these risks were even higher among the co-twins of children with CP born of twin pregnancies.

A Swedish population-based record linkage study that included 3997 patients with CP found that the risk of hospitalization for CP was 4.8 times greater than expected among the sibs of individuals with CP born of singleton pregnancies and 29 times greater than expected among the co-twins of individuals with CP born of twin pregnancies. [23]

These data indicate that genetic factors are often important in the aetiology of CP. The studies are compatible with a multifactorial mechanism or with genetic heterogeneity, with some cases resulting from genetic variants of major effect and others having a non-genetic cause. A multifactorial mechanism in some cases, various major genetic factors in other cases, and non-genetic causation in still others seems most likely.

Candidate Gene Association Studies

Association studies are used to identify genetic loci that predispose to or protect against the development of a disease. They are usually based on an assumption that the disease is multifactorial, i.e., caused by a complex combination of many different minor genetic and non-genetic factors.

Association studies of at least 160 different genetic variants in at least 60 candidate genes have been reported in patients with CP and corresponding control groups. Table 35.1 lists the genes and variants (mostly SNPs) that have been assessed in these studies. Most of these genetic loci were chosen for study because of their known involvement in blood clotting, vascular regulation or inflammation, processes that are thought to be important in the pathogenesis of, or physiological response to, perinatal intracranial bleeding [24–26]. These studies vary in terms of how the CP was defined, how the patients were ascertained, and what populations the patients represented [26, 27]. Most of the studies are small: the largest candidate gene association study of CP reported to date includes 763 cases [27], but many have fewer than 100 cases.

Although associations with CP have been reported with polymorphic genetic variants near or within a dozen different genes [27–38], none of these associations has been replicated in an independent investigation. Many of the reported associations are inverse or 'protective,' meaning that patients with the more frequent allele in the population are at higher risk, a counterintuitive observation. Even more associations have been observed in *ad hoc* subgroups of CP patients, but none of these has been independently replicated, and such observations are suspect for statistical reasons [24–26].

The inability to replicate candidate gene association studies is a common observation in complex diseases [39, 40]. Independent replication is essential because candidate disease association studies are often confounded by issues related to disease definition, patient ascertainment, population stratification, publication bias and statistical analysis. More recent genetic association studies of many complex diseases address these problems through genome-wide testing of tens of thousands or more SNPs in homogenous groups of thousands to tens of thousands of patients. We are not aware of any published genome-wide association studies of patients with CP [41], and it seems unlikely that this approach would be informative unless the known aetiological and pathogenetic heterogeneity of CP were taken into account in patient selection and data analysis.

Table 35.1 Loci that have been studied in CP candidate gene association studies [24, 27–31, 33–38, 81–88]

Gene	Locus	SNP	Location	Comment
Loci associated with blood clotting				
Annexin A5	*ANXA5*	rs1257049725	5′ UTR	
Factor II	*F2*	rs1799963	3′ UTR	Also known as *F2* (G20210A)
Factor V	*F5*	rs6025	Exon (missense)	Also known as factor V Leiden or *F5* (G1691A)
Factor VII	*F7*	rs6046	Exon (missense)	
		rs5742910	Upstream	
Fibrinogen beta chain	*FGB*	rs4220	Exon (missense)	
		rs1800790	Upstream	
Integrin subunit alpha 2	*ITGA2*	rs1062535	Exon (synonymous)	Also known as *ITGA2* (873G/ A)
Integrin subunit beta 3	*ITGB3*	rs5918	Exon (missense)	Also known as ITGB3 (leu33pro)
Methylenetetrahydrofolate reductase	*MTHFR*	rs1801133	Exon (missense)	Also known as *MTHFR* (C677T)
		rs1801131	Exon (missense)	Also known as *MTHFR* (A1298C)
		rs4846049	3′ UTR	
		rs1476413	Intron	
		rs9651118	Intron	
Plasminogen activator, tissue type	*PLAT*	rs2020918	Upstream	
Protein C receptor	*PROCR*	rs867186	Exon (missense)	Gene also known as *EPCR*
Serpin family B member 2	*SERPINB2*	rs6098	Exon (missense)	Also known as PAI_2–1
		rs6103	Exon (missense)	Also known as PAI_2–2
		rs6104	Exon (missense)	Also known as PAI2
Serpin family E member 1	*SERPINE1*	rs7242	3’ UTR	Also known as PAI1
		rs1799768	Upstream	Also known as PAI1
Thrombomodulin	*THBD*	rs1800576	Exon (missense)	
Tissue factor pathway inhibitor	*TFPI*	rs1189623	Intron	
Loci associated with inflammation				
Arachidonate 5-lipoxygenase activating protein	*ALOX5AP*	rs9551963	Intron	Also known as SG13S32
		rs17222842	Downstream	Also known as SG13S35
		rs4769874	Intron	
C-C motif chemokine ligand 18	*CCL18*	rs1102934	Upstream	
		rs2015086	Upstream	
		rs2015070	Intron	
		rs2735835	Intron	
		rs712044	Intron	
C-reactive protein	*CRP*	rs1205	3′ UTR	
C-X-C motif chemokine ligand 8	*CXCL8*	rs4073	Upstream	Gene also known as *IL-8*
Complement C3d receptor 2	*CR2*	rs3813946	5′ UTR	
		rs1048971	Exon (synonymous)	
		rs17615	Exon (missense)	
Complement factor H	*CFH*	rs1061170	Exon (missense)	
Intercellular adhesion molecule 1	*ICAM1*	rs1799969	Exon (missense)	
Interleukin 1 beta	*IL1B*	rs16944	Upstream	Also known as *IL1B* -511C/T
Interleukin 1 receptor antagonist	*IL1RN*	IL1RN, IVS2, 86-BP DUP	Intron	VNTR
Interleukin 10	*IL10*	rs1554286	Intron	SNP also in *IL19* 5’UTR
		rs1518111		
		rs3024490		
Interleukin 13	*IL13*	rs20541	Exon (missense)	
Interleukin 19	*IL19*	rs1800872	Intron	SNP also in *IL10*
		rs1800896	Intron	SNP also in *IL10*
		rs1800871	Intron	Also known as IL-10 -819

Table 35.1 (continued)

Gene	Locus	SNP	Location	Comment
Interleukin 1 beta	*IL1B*	rs1143623	Upstream	
		rs1143634	Exon (synonymous)	Also known as *IL1B* 3954
		rs4848306	Upstream	
Interleukin 4	*IL4*	rs2243250	Upstream	Also known as *IL4* -589C/T
Interleukin 6	*IL6*	rs1800795	Intron	Also known as IL-6 -174
		rs1554606	Intron	
		rs1800796	Intron	
		rs1800797	Intron	
		rs1880243	Upstream	Also known as IL-6 -7227
		rs2066992	Intron	
		rs10242595	Downstream	
		rs2069837	Intron	
		rs2069840	Intron	
		rs11766273	Downstream	
		rs12700386	Upstream	
Interleukin 6 receptor	*IL6R*	rs952146	Upstream	
		rs4075015	Intron	
		rs4537545	Intron	
		rs4601580	Intron	
		rs4845374	Intron	
		rs4845618	Intron	
		rs4845625	Intron	
		rs6687726	Intron	
		rs7549338	Intron	
Lymphotoxin alpha	*LTA*	rs1041981	Exon (missense)	
Mannose binding lectin 2	*MBL2*	rs5030737	Exon (missense)	Also known as MBL-52
		rs1800450	Exon (missense)	Also known as MBL-54
		rs1800451	Exon (missense)	Also known as MBL-57
		rs7096206	Intron	Also known as MBL −221
		rs7095891	Intron	Also known as MBL +4 C > T (P/Q)
		rs11003123	Intron	
		rs11003125	Intron	Also known as MBL −550
Secreted phosphoprotein 1	*SPP1*	rs2853744	Upstream	Gene also known as osteopontin (*OPN*)
		rs2853749	Intron	Gene also known as osteopontin (*OPN*)
		rs11728697	Exon (missense)	Gene also known as osteopontin (*OPN*)
		rs4754	Exon (missense)	Gene also known as osteopontin (OPN)
		rs1126616	Exon (synonymous)	Gene also known as osteopontin (*OPN*)
Selectin E	*SELE*	rs5361	Exon (missense)	Also known as *SELE* (ser128arg)
		rs5355	Exon (missense)	Also known as *SELE* (leu554phe)
Toll-like receptor 1	*TLR1*	rs5743551	5′ UTR	
Toll-like receptor 2	*TLR2*	rs4696480	Intron	
		rs5743708	Exon (missense)	
Toll-like receptor 4	*TLR4*	rs4986790	Exon (missense)	Also known as *TLR-4* (asp299gly)
		rs4986791	Exon (missense)	
Transforming growth factor beta 1	*TGFB1*	rs1800470	Exon (missense)	
		rs1800469	Upstream	Variant is 2 kb upstream of *TGFB1* and 500 bp downstream of *B9D2*
Tumour necrosis factor	*TNF*	rs1800629	Upstream	Also known as TNF-alpha −308 (G308A)
		rs1800610	Intron	Gene also known as TNF-alpha
		rs361525	Upstream	Also known as TNF-alpha-238
		rs1799964	Upstream	Also known as TNF-alpha-1031 T/C; SNP is also downstream of *LTA*
		rs1799724	Upstream	Also known as TNF-alpha-857 C/T; SNP is also downstream of *LTA*

(continued)

Table 35.1 (continued)

Gene	Locus	SNP	Location	Comment
Loci associated with vascular regulation				
Adrenoceptor beta	*ADRB2*	rs1042714	Exon (stop gain)	Also known as *ADRB2* (gln27glu)
		rs1042713	Exon (missense)	Also known as *ADRB2* (arg16gly)
		rs1042717	Exon (synonymous)	
Angiotensin	*AGT*	rs699	Exon (missense)	Also known as *AGT* (met235thr)
Angiotensin II receptor type 1	*AGTR1*	rs5186	3′ UTR	Also known as *AGTR1* (1166A/ C)
Natriuretic peptide A	*NPPA*	rs5063	Exon (missense)	Also known as *NPPA* (664G/A)
		rs5065	Exon (stop loss)	Also known as *NPPA* (2238 T/C)
Neuropeptide Y	*NPY*	rs16135	Intron	Also *LOC10798677* intron variant
		rs16476	Intron	Also *LOC10798677* intron variant
Nitric oxide synthase 1	*NOS1*	rs3782219	Intron	
		rs2293054	Exon (missense)	
		rs10774909	Intron	
		rs3741475	Exon (synonymous)	
		rs2682826	3′ UTR	
Nitric oxide synthase 2	*NOS2*	rs1137933	Exon (synonymous)	Gene also known as iNOS
		(CCTTT)n micro satellite	2.5 kb upstream	
Nitric oxide synthase 3	*NOS3*	rs1800779	Intron	Gene also known as eNOS
		rs3918226	Intron	Gene also known as eNOS
		rs1799983	Exon (missense)	Gene also known as eNOS
Sodium channel epithelial 1 subunit alpha	*SCNN1A*	rs5742912	Exon (missense)	Also known as *SCNN1A* (trp493arg)
		rs2228576	Exon (missense)	Also known as *SCNN1A* (ala663thr)
Other loci				
Adducin 1	*ADD1*	rs4961	Exon (missense)	
Apolipoprotein E	*APOE*	rs429358	Exon (missense)	
		rs7412	Exon (missense)	
		rs769446	Upstream	
		rs405509	Upstream	
		rs121918399	Exon (missense)	
		rs429358	Exon (missense)	
		rs190853081	Exon (missense)	
		ε2	Exon (missense)	Variant includes rs429358(T) and rs7412(t)
		ε3		Variant includes rs429358(T) and rs7412(C) (major alleles at both loci)
		ε4	Exon (missense)	Variant includes rs429358(c) and rs7412(C)
Autophagy related 5	*ATG5*	rs510432	Upstream	
		rs3804338	Intron	
		rs573775	Intron	
		rs2299863	Intron	
		rs6568431	Downstream	SNP also downstream of *PRDM1*
Autophagy related 7	*ATG7*	rs346078	Intron	
		rs1470612	Intron	
		rs11706903	Intron	
		rs2606750	Intron	
		rs2594972	Intron	
		rs4684787	Intron	
Collagen type IV alpha 1 chain	*COL4A1*	rs10492497	Intron	
		rs1961495	Intron	
		rs562992	Intron	
		rs1411040	Intron	
Collagen type IV alpha 2 chain	*COL4A2*	rs4773144	Intron	
		rs3809346	Intron	

Table 35.1 (continued)

Gene	Locus	SNP	Location	Comment
Cystathionine beta-synthase	*CBS*	rs5742905	Exon (missense)	
G protein subunit beta 3	*GNB3*	rs5443	Exon (synonymous)	Also known as *GNB3* (825C / T)
Glutamate decarboxylase 1	*GAD1*	rs379187	Intron	
		rs3791862	Intron	
		rs16858977	Intron	
Matrix metallopeptidase 2	*MMP2*	rs243865	Upstream	
Matrix metallopeptidase 3	*MMP3*	rs602128	Exon (missense)	
		rs3025058	Upstream	Also known as *MMP3* −1171 (5A/6A) (1 bp indel)
Oligodendrocyte transcription factor 2	*OLIG2*	rs6517135	Upstream	
		rs1005573	Intron	
		rs6517137	3′ UTR	
		rs9653711	Downstream	
Phosphodiesterase 4D	*PDE4D*	rs12188950	Intron	

Studies of Chromosomal Abnormalities and Genomic Copy Number Variants

Major genetic factors are those that are both necessary and sufficient to cause a particular disease in a patient. Clinically, major genetic causes of disease include inherited or de novo Mendelian disorders and chromosomal abnormalities. At a molecular level, the changes that cause genetic disease of major effect are alterations of nucleotide sequence, genomic copy number or genomic structure, alone or in combination. Most mutations that cause inherited or de novo Mendelian diseases are alterations of nucleotide sequence, usually single nucleotide variants. Alterations of genomic copy number or structure are conventionally called 'chromosomal abnormalities' because microscopic (cytogenetic) analysis has been used to identify them for more than 60 years. However, most disease-causing genomic alterations are too small to be visualized under the light microscope and require molecular techniques such as chromosomal microarray analysis or genome sequencing for detection.

Anecdotal reports of patients with CP and various chromosomal abnormalities have occasionally appeared in the medical literature [42–45], and a few patients with segmental gain or loss of genomic material large enough to be seen cytogenetically and a 'cerebral palsy' phenotype are reported in the DECIPHER or ClinVar databases (Table 35.2). However, we are not aware of any study describing the results of routine cytogenetic testing in a large series of patients with CP.

In a study of data from eleven European CP registries, 13 (0.3%) of 4584 children with CP born between 1976 and 1996 were reported to have chromosomal abnormalities detected by cytogenetic analysis [46]. This must be a minimal estimate because the techniques available for identifying genomic imbalance were much less sensitive at that time than they are today and because cytogenetic analysis was infrequently done on children with CP, which was usually assumed to have been caused by perinatal anoxia or intracranial bleeding.

Segmental gains or losses of genomic material are usually called 'deletions' or 'duplications' if they can be demonstrated under the microscope and 'copy number variants' (CNVs) if they are smaller (generally <10 Mb) and require the use of molecular techniques, such as chromosomal microarray analysis (CMA) or exome sequencing, to be detected. Much smaller (1–50 bp) genomic gains or losses that can only be identified by sequencing are called 'indels'.

Variability is a normal feature of the human genome. The nucleotides of two unrelated people differ by about 1% of their total nucleotide sequence or content and by more than 20,000 CNVs, on average [47]. Most of these variants occur as polymorphisms in the general population and are inherited from one parent or the other, and most are thought to be unrelated to the occurrence of CP or any other disease. A small fraction of the genomic variants in each of us arise de novo as a result of new mutations.

There are two critical steps in identifying disease-causing CNVs in patients with CP. The first is recognition of the genomic variant, which is usually done by CMA or DNA sequencing, and the second is determining that the variant is, in fact, capable of causing disease. Rare CNVs that are both necessary and sufficient to cause a genetic disease are classified as 'pathogenic' or 'likely pathogenic' according to standard laboratory criteria [48]. Most CNVs that are unrelated to the occurrence of a genetic disease can be classified as 'benign' or 'likely benign'. We are unable to determine whether some other CNVs have an effect on the phenotype – such variants are classified as being of 'uncertain significance'.

A few individual patients with CP and other neurodevelopmental abnormalities who were found to have apparently

Table 35.2 CNVs reported in ClinVar or DECIPHER in patients whose phenotype includes CP

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
ClinVar variation ID	154737	3.4 Mb copy number gain	1q21.1–21.2	Pathogenic	Autism, delayed speech and language development, pituitary dwarfism, hyperpigmentation of the skin, muscular hypotonia, global developmental delay, failure to thrive, morphological abnormality of the central nervous system, delayed gross motor development, short stature, attention deficit hyperactivity disorder, delayed fine motor development, cerebral palsy, behavioural abnormality	
ClinVar variation ID	144454	4.9 Mb copy number gain	2p25.3–25.2	Pathogenic	Cerebral palsy	
ClinVar variation ID	154597	7.5 Mb copy number loss	2q23.3–24.2	Pathogenic	Failure to thrive, cerebral palsy	
DECIPHER patient	283420	751.6 kb copy number loss	4p13	Pathogenic	Cerebral palsy, congenital hypothyroidism, hemiplegia, intrauterine growth retardation	
DECIPHER patient	283426	446.7 kb copy number gain	5p15.2	Pathogenic	Cerebral palsy, congenital hypothyroidism, hemiplegia, intrauterine growth retardation	
ClinVar variation ID	442344	9.9 Mb copy number gain (4 copy)	5q12.1–13.2	Likely pathogenic	Global developmental delay, hypertonia, abnormal heart morphology, abnormal facial shape, cerebral palsy	
DECIPHER patient	283424*	136 bp copy number loss	5q21.1 (*SLCO6A1* gene)	Pathogenic	Anxiety, autism, cerebral palsy, global developmental delay, intellectual disability, mild, intracranial hemorrhage, periodontitis, tetraplegia	
ClinVar variation ID	443701	5.2 Mb copy number loss	6q14.1–14.3	Likely pathogenic	Triangular face, upslanted palpebral fissure, seizures, absent speech, abnormal facial shape, cerebral palsy	
ClinVar variation ID	154347	1.6 Mb copy number loss	7q11.23	Pathogenic	Cerebral palsy, hearing impairment, microcephaly	Copy number loss does not overlap region associated with NF1 microdeletion syndrome
DECIPHER patient	283422†	567.8 kb copy number gain	7q21.13	Pathogenic	Cerebral palsy, cerebral visual impairment, generalized myoclonic seizure, global developmental delay, spastic diplegia	
DECIPHER patient	388863	**11.8 Mb copy number gain**	**7q32.1-q35**	Likely pathogenic	Abnormal heart morphology, autistic behaviour, cerebral palsy, seizure	
DECIPHER patient	355383‡	273.3 kb copy number gain	7q34	Likely pathogenic	Athetoid cerebral palsy, delayed speech and language development, generalized hypotonia, global developmental delay, growth delay	
DECIPHER patient	355383‡	3.54 Mb copy number gain (4 copy)	7q34-7q35	Likely pathogenic	Athetoid cerebral palsy, delayed speech and language development, generalized hypotonia, global developmental delay, growth delay	

Table 35.2 (continued)

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
DECIPHER patient	283421[§]	219.4 kb copy number gain	8p23.1	Pathogenic	Cerebral palsy, hemiplegia, hypoplasia of the corpus callosum, periventricular leukomalacia, porencephalic cyst	
ClinVar variation ID	441537	4.7 Mb copy number loss	10p15.3–15.1	Pathogenic	Cerebral palsy	
DECIPHER patient	283429	234.3 kb copy number gain	10q26.13	Pathogenic	Cerebral palsy, global developmental delay, hemiplegia	
ClinVar variation ID	155548	6.8 Mb copy number loss	10q26.2–26.3	Pathogenic	Cerebral palsy	
ClinVar variation ID	443505	6.4 Mb copy number gain	10q26.2–26.3	Pathogenic	Cerebral palsy	
ClinVar variation ID	154432	**14.4 Mb copy number loss**	**11p15.1–13**	Pathogenic	Cerebral palsy, cataract, global developmental delay, hydrocephalus, aniridia, microcephaly	WAGR 13p13 deletion occupies 651 kb within this much larger deletion
DECIPHER patient	283422[†]	386.6 kb copy number loss	12p12.2	Pathogenic	Cerebral palsy, cerebral visual impairment, generalized myoclonic seizure, global developmental delay, spastic diplegia	
DECIPHER patient	283425	211.2 kb copy number gain	14q23.1	Pathogenic	Cerebral palsy, spastic diplegia	
DECIPHER patient	283421[§]	534.6 kb copy number gain	15q11.2	Pathogenic	Cerebral palsy, hemiplegia, hypoplasia of the corpus callosum, periventricular leukomalacia, porencephalic cyst	
DECIPHER patient	341043	467.9 kb copy number loss	15q11.2	Likely pathogenic	Bicuspid aortic valve, cerebral palsy, intellectual disability, moderate	
ClinVar variation ID	58,073	5.0 Mb copy number gain	15q11.2–13.1	Pathogenic	Seizure, cerebral palsy	
ClinVar variation ID	154724	7.2 Mb copy number gain (4 copy)	15q11.2–13.2	Pathogenic	Seizures, global developmental delay, abnormal heart morphology, cerebral palsy	
ClinVar variation ID	154725	1.9 Mb copy number gain	15q13.2–13.3	Pathogenic	Seizures, global developmental delay, abnormal heart morphology, cerebral palsy	
ClinVar variation ID	144213	3.4 Mb copy number gain	17p11.2	Pathogenic	Cerebral palsy	Duplication compatible with Potocki-Lupski syndrome. The usual features of this syndrome are mild developmental delay/ intellectual disability, autistic features, attention-deficit hyperactivity disorder, failure to thrive in early childhood, dysmorphic facial features and sometimes structural cardiovascular abnormalities
DECIPHER patient	283423	4.5 kb copy number loss	17p11.2 (*COPS3* gene)	Pathogenic	Cerebral palsy, delayed gross motor development, generalized myoclonic seizure, hemiplegia, periventricular leukomalacia, porencephalic cyst	
ClinVar variation ID	153240	1.4 Mb copy number gain	17p12	Pathogenic	Areflexia, autism, gastroesophageal reflux, aortic aneurysm, peroneal muscle atrophy, cerebral palsy	
ClinVar variation ID	59561	382.8 kb copy number loss	17p13.3	Pathogenic	Seizure, cerebral palsy	

(continued)

Table 35.2 (continued)

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
DECIPHER patient	283428	**68.7 Mb copy number loss**	**17p13.3-q25.1**	Pathogenic	Cerebral palsy, generalized myoclonic seizure, intellectual disability, moderate, polymicrogyria, tetraplegia	
ClinVar variation ID	443555	1.4 Mb copy number gain	17q12	Likely pathogenic	Muscular hypotonia, failure to thrive, respiratory failure, short stature, hypoxemia, cerebral palsy	
ClinVar variation ID	155320	483.7 kb copy number loss	17q21.31	Pathogenic	Autistic behaviour, intellectual disability, seizures, abnormality of the corpus callosum, cortical dysplasia, cerebral palsy	Deletion compatible with Koolen de Vries syndrome. The usual features of this syndrome include intellectual disability, hypotonia, seizures, structural brain abnormalities, and autistic behaviour. Other findings include dysmorphic facial features, cardiovascular malformations, renal anomalies and abnormalities of the skin and hair. Cerebral palsy not a recognized feature of this syndrome
DECIPHER patient	283427	612.3 kb copy number gain	17q25.3	Pathogenic	Autism, cerebral palsy, generalized myoclonic seizure, global developmental delay, hemiplegia, intellectual disability, mild	
DECIPHER patient	283424*	64.5 kb copy number gain	18p11.21	Pathogenic	Anxiety, autism, cerebral palsy, global developmental delay, intellectual disability, mild, intracranial haemorrhage, periodontitis, tetraplegia	
ClinVar variation ID	58724	145.0 kb copy number gain	18p11.32	Pathogenic	Autism, cerebral palsy, gait disturbance	
ClinVar variation ID	812928	9.5 kb copy number loss	19p13.12	Likely pathogenic	Cerebral palsy; global developmental delay; visual impairment	
ClinVar variation ID	442027	228.6 kb copy number loss	20q13.33	Pathogenic	Abnormality of vision, intellectual disability, seizures, abnormalfacial shape, scoliosis, short stature, cerebral palsy	
DECIPHER patient	303619	2.1 Mb copy number loss	22q11.21	Pathogenic	Abnormal facial shape, broad forehead, cerebral palsy, clinodactyly of the fourth toe, global developmental delay, microcephaly, rheumatoid arthritis, ventricular septal defect	
ClinVar variation ID	57671	6.7 Mb copy number loss	22q13.31–13.33	Pathogenic	Cerebral palsy, gait disturbance, autism	The 142 kb critical region of the Phelan-Mcdermid syndrome is included at one end of this much larger deletion. The usual features of this syndrome are moderate to severe intellectual disability with particular difficulty in speech, autistic behaviour, seizures, tall stature and dysmorphic facial features. Some affected children have been diagnosed with cerebral palsy because they have neonatal hypotonia, delayed walking and unsteady gait

Table 35.2 (continued)

Identifier	Number	Description	Location	Interpretation	Clinical features	Comments
DECIPHER patient	283424*	169.2 kb copy number gain	22q13.33 (*MC2R* gene)	Pathogenic	Anxiety, autism, cerebral palsy, global developmental delay, intellectual disability, mild, intracranial haemorrhage, periodontitis, tetraplegia	
DECIPHER patient	284245	29.0 kb (male)	Xp11.4 (*OTC* gene)	Likely pathogenic	Cerebral palsy, episodic ammonia intoxication	
ClinVar variation ID	154959	1.6 Mb copy number loss (presumed male)	Xp22.31	Pathogenic	Autism, dystonia, cerebral palsy, cortical visual impairment	
ClinVar variation ID	443632	2.0 Mb copy number loss (presumed male)	Xq26.2–26.3	Pathogenic	Intellectual disability, seizures, cerebral palsy	
DECIPHER patient	388870	**13.3 Mb (male)**	**Xq27.1-q28**	Pathogenic	Cerebral palsy, intellectual disability, moderate, short stature	
ClinVar variation ID	154936	470.7 kb copy number loss (presumed male)	Xq28	Pathogenic	Seizure, cerebral palsy, global developmental delay	
ClinVar variation ID	154935	6.5 Mb copy number gain (presumed male)	Xq28	Pathogenic	Cerebral palsy, global developmental delay, seizure	

Data are from https://www.ncbi.nlm.nih.gov/clinvar/ or https://www.deciphergenomics.org/. Copy number changes highlighted in **bold font** are large enough to be detected by routine cytogenetic analysis. DECIPHER patients with the same superscript symbol (*, †, ‡, §) are individuals who are reported to have two or more pathogenic/likely pathogenic copy number changes

disease-causing CNVs have been described in the medical literature [49–51], but it is impossible to determine if the co-occurrence of CP and the CNV in these anecdotal cases reflects a causal or coincidental relationship. Dozens of patients with various pathogenic or likely pathogenic CNVs and CP are listed in the ClinVar [52] or DECIPHER [53] databases (Table 35.2). Almost all of these patients have other neurodevelopmental conditions in addition to CP, and a few are reported also to have malformations of other organ systems or dysmorphic features. Most of the CNVs seen in these patients are unique; very few are recurrent copy number changes that are recognized causes of specific genetic syndromes (Table 35.2).

In one remarkable family, nine individuals with spastic quadriplegia and intellectual disability where found by molecular techniques to carry a 225 kb copy number loss of chromosome 9p24.3 that includes the *KANK1* (*ANKRD15*) gene [54]. This CNV, which was transmitted through at least four generations, is incompletely penetrant but appears to have caused the CP in affected family members. Individuals with various *KANK1* copy number losses from other families do not usually have CP [55].

DECIPHER provides a list of 66 genetic syndromes that are caused by CNVs [56]. None of these conditions includes CP as a cardinal feature. However, some children with the Phalen-McDermid (22q13 deletion) syndrome are diagnosed with cerebral palsy because they have neonatal hypotonia, delayed walking and unsteady gait [57].

Seven patient series have determined the frequency of CNVs among individuals with CP (Table 35.3). Most of these studies found that relatively few (0–6%) of the CP patients had disease-causing CNVs. One exception was a series of 52 patients with disabling non-progressive pyramidal and/or extra pyramidal signs beginning before 3 years of age and no periventricular leukomalacia or spinal cord lesions and no history of hypoxic ischemic encephalopathy, brain infarction, encephalitis or head trauma [58]. Sixteen pathogenic or likely pathogenic CNVs were found in 16 (31%) of these atypical CP patients. Patients in the other series who had disease-causing CNVs often had other neurodevelopmental disorders such as intellectual disability, autism or epilepsy, and some had structural malformations of the brain or other organ systems. Unfortunately, however, the clinical descriptions, apart from their CP, reported for patients in these series are limited.

Most disease-causing CNVs in CP patients occur de novo, rather than being inherited from one of the parents. This is true of disease-causing CNVs in other neurodevelopmental disorders as well [59, 60].

The pathogenic/likely pathogenic CNVs reported in these CP patient series involved many different chromosomal regions. This observation is consistent with the het-

Table 35.3 Studies of disease-causing CNVs in CP patient series

Study	CP patients studied	Patient group studied	Method of CNV testing	Diagnostic rate	Comments	Pathogenic/likely pathogenic CNVs observed
McMichael (2014) [75]	50	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	CMA	No pathogenic or likely pathogenic CNVs found	14 rare CNVs found in 10 cases; no proven de novo CNVs; all classified as VUS by FRANKLIN.	None
Segel (2015) [58]	52	CP with undetermined aetiology and disabling non-progressive pyramidal and/or extra pyramidal signs; periventricular leukomalacia, perinatal anoxia excluded	CMA	16 pathogenic or likely pathogenic CNVs found in 16 patients (31%)	9 de novo pathogenic/likely pathogenic CNVs, 7 pathogenic/likely pathogenic CNVs inherited from a parent; 6/16 pathogenic or likely pathogenic CNVs explained the CP phenotype. Most individuals with pathogenic or likely pathogenic CNVs also had ID and/or epilepsy	154 kb del(1)(p21.3) 5.21 Mb del(2)(p23.1p22.2) 862 kb dup(2)(q13) 3.46 Mb del(5)(q14.3) 152 kb del(7)(q31.1) 226 kb del(9)(p24.3) 147 kb del(9)(q34.13q34.2) 11.15 Mb del(14)(q12q21.2) 3.23 Mb del(14)(q32.31q32.33) 387 kb dup(17)(p11.2) 445 kb dup(18)(p11.21) 1.96 Mb del(19)(q13.12) 679 kb dup(20)(p12.3p12.2) 2.82 Mb del(22)(q11.21) 4.29 Mb del(X)(p11.23p11.22)(male patient) 298 kb trp(X)(q28)(male patient)
Oskoui (2015) [76]	147	Children with CP diagnosed by specialist physicians at paediatric rehabilitation centres	CMA	8 pathogenic or likely pathogenic CNVs found in 6 patients (4.1%)	All but one pathogenic/likely pathogenic CNVs thought to be de novo but in two cases involving 4 CNVs, one of the parents may have carried a balanced reciprocal translocation	2.08 Mb del(1)(q21.1q21.2) 73.97 Mb dup(2)(p25.3p13.1) and 30.97 Mb del(X)(p22.33p21.2) (female patient) 12.11 Mb dup(2)(p25.3p24.3) 25.49 Mb del(4)(p16.3p15.2) and 8.10 Mb del(9)(p24.3p24.1) 5.79 MB del(15)(q11.2q13.1) 2.76 Mb dup(22)(q13.31)
Zarrei, (2018) [89]	97	Patients with hemiplegic CP	CMA	5 pathogenic or likely pathogenic CNVs found in 4 patients (4.1%)	4 de novo pathogenic/likely pathogenic CNVs, 1 likely pathogenic CNV inherited from a parent;	1.40 Mb del(17)(p12) 2.55 Mb dup(22)(q11.21) 155.27 Mb dup(X)(p22.33q28) (male patient, Klinefelter syndrome) 84.89 Mb del(X)(q13.1q28) and 70.38 Mb dup(X)(p22.33q13.1) (female patient)
Takezawa [65]	17	CP patients born at term with no apparent acquired cause of CP and no typical findings on brain MRI	CMA	Pathogenic CNV found in 1 patient (6%)	One patient with CP, ID, epilepsy, and microcephaly found to have 47, XXY	155.27 Mb dup(X)(p22.33q28) (male patient, Klinefelter syndrome)

Table 35.3 (continued)

Study	CP patients studied	Patient group studied	Method of CNV testing	Diagnostic rate	Comments	Pathogenic/likely pathogenic CNVs observed
Corbett (2018) [77]	136 cases not previously studied	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	Trio exome sequencing with bioinformatic analysis for CNVs	9 pathogenic or likely pathogenic CNVs found in 7 patients (5.1%)	8 de novo pathogenic/likely pathogenic CNVs, 1 pathogenic CNV for which both parents were not tested. In 4/9 patients with pathogenic or likely pathogenic CNVs the copy number change was thought to explain the CP phenotype	4.09 Mb dup(1)(q21.1) 7.51 Mb dup(1)(q43q44) and 50.35 Mb del(X)(p22.33p11.22) (unbalanced reciprocal translocation in female patient) 2.55 Mb del(2)(p25.3) and 8.01 Mb dup(20)q13.2q13.33) (unbalanced reciprocal translocation) 519 kb del(3)(p22.3) 726 kb del(16)(p11.2-p12.2) 2.82 Mb del(22)(q11.21) 2.81 Mb dup(22q11)
Rosello, (2020) [67]	20	Children with CP diagnosed by standard criteria who do not have a multiple congenital anomaly syndrome, ataxic CP, progressive encephalopathy, or neuroradiological findings of hypoxic-ischemic encephalopathy, periventricular leukomalacia, cerebral malformation, or leukoencephalopathy	CMA	No pathogenic or likely pathogenic CNVs found		

CNV copy number variant, *CMA* chromosomal microarray analysis. FRANKLIN <https://franklin.genoox.com/clinical-db/home> is a website that provides on-line assessment of genomic variants using the ACMG criteria

erogeneous genetic aetiology of CP discussed above. However, it is interesting that some specific CNVs were reported in patients in two different series: del(22)(p11.21), dup(22)(p11.21), and duplication of the entire X chromosome in males (Table 35.3). The clinical syndromes associated with these CNVs (velocardiofacial/Di George syndrome, 22q11 duplication syndrome and Klinefelter syndrome, respectively) are well characterized, but cerebral palsy is not a usual feature of any of them.

Studies of Single Nucleotide Variants and Indels

Each of us has 4,000,000 to 5,000,000 single nucleotide variants (SNVs) and 700,000 to 800,000 indels (insertions or deletions of 1 to 50 nucleotides) in comparison to the reference human genome sequence [47]. Such 'small' alterations of nucleotide sequence are more frequent major causes of genetic disease than larger changes such as chromosomal abnormalities or genomic CNVs. Although small sequence variants can cause Mendelian diseases, most SNVs and indels are simply genomic differences that are transmitted from generation to generation without any apparent effect on the phenotype. SNVs and indels also arise by new mutation in every person. Most of these de novo changes, like the majority of inherited variants, occur outside of the genes and have no effect on the phenotype. However, if a mutation affects a gene, the change may abrogate or alter the gene's normal function.

Although many different technologies were used to identify disease-causing SNVs and indels in the past, the advent of accurate, rapid, and increasingly cost-effective 'next-generation' or 'second-generation' DNA sequencing has made it routinely possible to test panels of hundreds or thousands of genes, all protein-coding segments of every gene (the 'whole exome'), or all of a person's DNA (the 'whole genome') at once. Rare SNVs or indels that are both necessary and sufficient to cause a genetic disease are conventionally classified as 'pathogenic' or 'likely pathogenic' variants according to standard laboratory criteria [61]. Most SNVs or indels have no influence on the phenotype and can be classified as 'benign' or 'likely benign', but some variants cannot easily be interpreted and must be classified as variants of uncertain significance. Recognizing the one or two genomic variants that cause a Mendelian disease in an affected person's exome or genome sequence data requires sophisticated bioinformatics and clinical analysis of the results.

OMIM [62], an online catalogue of human genes and genetic phenotypes, lists 58 genetic diseases that may pres-

ent as cerebral palsy (Table 35.4). These Mendelian disorders are caused by alterations of 54 different genes. It is important to note that other neurodevelopmental abnormalities occur in all of these diseases and some have multisystem manifestations. Some are progressive and can be recognized as being different from typical CP once this becomes apparent clinically.

The results of exome sequencing have been reported in more than 350 CP or atypical CP patients (Table 35.5). The largest published series, which was recently reported by Jin

Table 35.4 Mendelian conditions that may present with cerebral palsy. Data are from Online Mendelian Inheritance in Man <https://www.omim.org/>

MIM number	Disease	Associated gene	Inheritance	CP phenotypes
201450	Acyl-CoA dehydrogenase, medium-chain, deficiency of	ACADM	Autosomal recessive	Cerebral palsy; hypotonia
617008	Cerebral palsy, spastic quadriplegic, 3	ADD3	Autosomal recessive	Spastic quadriplegia; spastic diplegia
614066	Spastic paraplegia 47, autosomal recessive	AP4B1	Autosomal recessive	Spasticity; inability to walk unaided
613744	Spastic paraplegia 51, autosomal recessive	AP4E1	Autosomal recessive	Spastic quadriplegia
612936	Spastic paraplegia 50, autosomal recessive	AP4M1	Autosomal recessive	Spastic quadriplegia
614067	Spastic paraplegia 52, autosomal recessive	AP4S1	Autosomal recessive	Spasticity; loss of ability to walk
207800	Argininemia	ARG1	Autosomal recessive	Spastic quadriplegia
615926	Webb-Dattani syndrome	ARNT2	Autosomal recessive	Spasticity; cerebral palsy
271900	Canavan disease	ASPA	Autosomal recessive	Initial hypotonia, followed by spasticity
182600	Spastic paraplegia 3, autosomal dominant	ATL1	Autosomal dominant	Lower limb spasticity; lower limb weakness; spastic gait
208900	Ataxia-telangiectasia	ATM	Autosomal recessive	Cerebellar ataxia; choreoathetosis; dystonia
615474	Primary aldosteronism, seizures and neurologic abnormalities	CACNA1D	Autosomal dominant	Cerebral palsy; movement disorder
618522	Mental retardation, autosomal dominant 59	CAMK2G	Autosomal dominant	Hyptonia; cerebral palsy
175780	Brain small vessel disease 1 with or without ocular anomalies	COL4A1	Autosomal dominant	Infantile hemiparesis; hemiplegia; tetraparesis; spasticity; limb dystonia
617976	Developmental and epileptic encephalopathy 63	CPLX1	Autosomal recessive	Hyptotonia; inability to walk
250800	Methemoglobinemia, type II	CYB5R3	Autosomal recessive	Hypertonia; spasticity
300958	Intellectual developmental disorder, X-linked, syndromic, snijders blok type	DDX3X	X-linked dominant or recessive	Dystonia; dyskinesia; spasticity; wide-based gait
310200	Muscular dystrophy, duchenne type	DMD	X-linked recessive	Hypotonia; waddling gait
614219	Adams-Oliver syndrome 2	DOCK6	Autosomal recessive	Hypotonia; spasticity; cerebral palsy
158600	Spinal muscular atrophy, lower extremity-predominant, 1, autosomal dominant	DYNC1H1	Autosomal dominant	Difficulty running and climbing stairs; waddling gait
617046	Spastic paraplegia 77, autosomal recessive	FARS2	Autosomal recessive	Spastic paraplegia
618557	Developmental and epileptic encephalopathy 78	GABRA2	Autosomal dominant	Hypotonia, axial; hypertonia, limb; choreiform movements; spasticity
603513	Cerebral palsy, spastic quadriplegic, 1	GAD1	Autosomal recessive	Spastic diplegia, symmetric; spastic quadriplegia
619124	Developmental and epileptic encephalopathy 89	GAD1	Autosomal recessive	Axial hypotonia; peripheral hypertonia; peripheral spasticity; spastic quadriplegia; dystonia; inability to walk
231670	Glutaric acidemia I	GCDH	Autosomal recessive	Dystonia; hypotonia; choreoathetosis
128230	Dystonia, dopa-responsive	GCH1	Autosomal dominant	Postural dystonia; action dystonia; gait abnormalities; gait ataxia

Table 35.4 (continued)

MIM number	Disease	Associated gene	Inheritance	CP phenotypes
603903	Sickle cell Anaemia	HBB	Autosomal recessive	Stroke; cerebral palsy
300322	Lesch-Nyhan syndrome	HPRT1	X-linked recessive	Hypotonia; spasticity; dystonia; choreoathetosis
117360	Spinocerebellar ataxia 29	ITPR1	Autosomal dominant	Broad-based gait; limb ataxia
206700	Gillespie syndrome	ITPR1	Autosomal recessive	General hypotonia; ataxia
160120	Episodic ataxia, type 1	KCNA1	Autosomal dominant	Ataxia, episodic; leg stiffness; spastic gait
605259	Spinocerebellar ataxia 13	KCNC3	Autosomal dominant	Cerebellar ataxia; hypotonia; inability to run
615834	Mental retardation, autosomal dominant 26	KIAA0442	Autosomal dominant	Hypertonia; stiff movements
210200	3-Methylcrotonyl-CoA carboxylase 1 deficiency	MCCC1	Autosomal recessive	Cerebral palsy; hypotonia
251280	Diencephalic-mesencephalic junction dysplasia syndrome 1	PCDH12	Autosomal recessive	Spastic quadriplegia; axial hypotonia; inability to stand or walk; dystonia
312170	Pyruvate dehydrogenase E1-alpha deficiency	PDHA1	X-linked recessive	Hypotonia; ataxia, episodic; choreoathetosis; dystonia
245349	Pyruvate dehydrogenase E3-binding protein deficiency	PDHX	Autosomal recessive	Hypotonia, neonatal; spastic paraplegia; spastic quadriplegia; ataxia; dystonia
312080	Pelizaeus-Merzbacher disease	PLP1	X-linked recessive	Hypotonia; ataxia; spasticity; dystonia; choreoathetosis
312920	Spastic paraplegia 2, X-linked	PLP1	X-linked recessive	Lower limb weakness; lower limb spasticity; spastic gait; ataxia
612304	Thrombophilia due to protein C deficiency, autosomal recessive	PROC	Autosomal recessive	Spastic cerebral palsy
128200	Episodic kinesigenic dyskinesia 1	PRRT2	Autosomal dominant	Dyskinesia, episodic; choreoathetosis, episodic; dystonia, episodic
600118	Warburg micro syndrome 1	RAB3GAP1	Autosomal recessive	Hypotonia; spastic diplegia
610181	Aicardi-Goutieres syndrome 2	RNASEH2B	Autosomal recessive	Spastic paraplegia; dystonia
616260	Tenorio syndrome	RNF125	Autosomal dominant	Hypotonia; abnormal gait; cerebral palsy
300523	Allan-Herndon-Dudley syndrome	SLC16A2	X-linked recessive	Hypotonia, proximal; spastic paraplegia; spastic quadriplegia; ataxia; inability to stand or walk
618973	Neurodegeneration, infantile-onset, biotin-responsive	SLC5A6	Autosomal recessive	Hypertonia; inability to walk; ataxia; dyskinetic movements; spasticity
613135	Parkinsonism-dystonia, infantile, 1	SLC6A3	Autosomal recessive	Truncal hypotonia; limb dystonia; dyskinesia; hypertonicity
609136	Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome and Hirschsprung disease	SOX10	Autosomal dominant	Spastic paraparesis, spastic quadriplegia, ataxia
612716	Dystonia, dopa-responsive, due to sepiapterin reductase deficiency	SPR	Autosomal recessive	Dystonia; spasticity; axial hypotonia; choreoathetosis; ataxia
600224	Spinocerebellar ataxia 5	SPTBN2	Autosomal dominant	Cerebellar ataxia
615386	Spinocerebellar ataxia, autosomal recessive 14	SPTBN2	Autosomal recessive	Gait ataxia; spasticity
605407	Segawa syndrome, autosomal recessive	TH	Autosomal recessive	Truncal hypotonia; limb dystonia; hypokinesia
618730	Neurodevelopmental disorder with microcephaly, cortical malformations and spasticity	TMX2	Autosomal recessive	Inability to walk; spasticity; spastic tetraplegia
618201	Developmental and epileptic encephalopathy 68	TRAK1	Autosomal recessive	Hypotonia; spasticity
225750	Aicardi-Goutieres syndrome 1	TREX1	Autosomal recessive or dominant	Tetraplegic spasticity; truncal hypotonia; dystonia
105830	Angelman syndrome	UBE3A	Autosomal dominant	Ataxia with jerky arm movements; wide-based gait; clumsiness, unsteadiness
224050	Cerebellar ataxia, mental retardation and Dysequilibrium syndrome 1	VLDLR	Autosomal recessive	Cerebellar ataxia; broad-based gait; quadrupedal gait
314580	Wieacker-Wolff syndrome	ZC4H2	X-linked recessive	Hypotonia; dystonia; spasticity

MIM Mendelian inheritance in man

Table 35.5 Studies of disease-causing SNVs and indels in CP patient series

Study	Number of CP patients studied	CP group studied	Sequencing performed	Diagnostic rate	Comments
Schnekenburg et al. (2015) [64]	10	Ten patients with congenital ataxia	Trio exome sequencing or 118 gene panel sequencing	3 pathogenic or likely pathogenic SNVs found in 10 patients (30%)	All three patients had de novo autosomal dominant conditions
Takezawa et al. (2018) [65]	17	CP patients who were born at term and do not have an apparent acquired cause of CP or findings characteristic of CP on brain MRI	Trio exome sequencing	10 pathogenic or likely pathogenic SNVs or indels found in 9 patients (53%)	Two patients had an autosomal recessive disease—one was homozygous and the other compound heterozygous. The other pathogenic or likely pathogenic variants were all heterozygous and de novo
Zhu et al. (2018) [90]	9	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	Singleton exome sequencing	0	No variants classified as pathogenic or likely pathogenic using current standards
Matthews et al. (2019) [66]	50 individuals in 49 families	Children with impaired motor function of unknown cause within the first year of life and one or more of the following: Severe intellectual disability, progressive neurological deterioration, other neurological abnormalities, multiorgan disease, congenital anomalies outside of the CNS, abnormal neurotransmitter profile, positive family history or brain imaging findings not typical for CP	Trio exome sequencing	Pathogenic or likely pathogenic variants found in 21 (43%) of 49 probands	Eleven patients had de novo autosomal dominant variants. Five patients had autosomal recessive diseases. One was a homozygote, and the others were compound heterzygotes. Five patients, two of them females, had X-linked diseases. The authors suggest that VUSs or likely pathogenic variants in genes that do not have an established association with CP found in 11 other patients may also be disease-causing
Van Eyk et al. (2019) [91]	271	Children with CP diagnosed by specialist physicians using standard criteria (non-progressive)	112 gene panel	Pathogenic or likely pathogenic variants found in 5 (1.8%) of 271 patients	Three patients had an autosomal dominant disease; one case was de novo and the parents were not both studied in the other two cases. One homozygous variant was found in a patient with an autosomal recessive disease, and one male had a variant for an X-linked recessive disease
Jin et al. (2020) [63]	250	Patients with CP defined as a non-progressive developmental disorder of movement and/or posture with onset before age 2 years. Cases with chromosomal anomalies, pathogenic CNVs, other clinically or molecularly diagnosed syndromes, mitochondrial disorders or traumatic brain injuries were excluded	Trio exome sequencing	The authors estimate that at least 14% of CP cases studied can be attributed to a disease-causing SNV or indel	The authors estimate that 11.9% of the CP cases studied can be attributed to a damaging de novo mutation and that 2.1% can be attributed to damaging recessive genotypes. These estimates are based on case-control analyses rather than on classification of individual variants with respect to pathogenicity, as was done in all other studies included in this table. Data in this study include 91 patients previously reported by McMichael et al., 2015 [92].
Rosello et al. (2020) [67]	20	Children with CP diagnosed by standard criteria who do not have a multiple congenital anomaly syndrome, ataxic CP, progressive encephalopathy, or neuroradiological findings of hypoxic-ischemic encephalopathy, periventricular leukomalacia, cerebral malformation or leukoencephalopathy	Trio exome sequencing	13 pathogenic or likely pathogenic SNVs found in 11 patients (55%)	Three patients had an autosomal recessive disease -- one was a homozygote and two were compound heterozygotes. One male patient had X-linked disease variant. The other disease-causing variants were all de novo autosomal dominants

and associates [63], includes 250 patients with CP defined by standard clinical criteria. This study was performed to explore genetically mediated disease mechanisms in CP, and SNVs and indels were assessed using case-control analyses of patient groups rather than by classification of variants for pathogenicity in each individual patient, as is done when exome sequencing is used clinically. On the basis of their analysis, Jin and associates [63] estimated that CP can be attributed to disease-causing SNVs or indels in at least 14% of patients. This clearly is a minimal estimate of the rate of disease-causing small nucleotide sequence changes among patients with conventionally defined CP [63]. Substantially higher proportions of patients with disease-causing SNVs or indels were observed in the patient series reported by Schnekenberg et al. [64], Takezawa et al. [65], Matthews et al. [66] or Rosello et al. [67], but all of these studies are much smaller and many of the patients included have an atypical form of CP (Table 35.5).

Disease-causing SNVs or indels reported in patients with CP or atypical CP involve 54 different genes (Table 35.6). The diseases caused by genetic alterations at some of these genetic loci are recognized as being associated with clinical features of CP, but 42 (78%) of the genes are *not* included in the list of

Table 35.6 Mendelian causes of CP reported in series studied by exome sequencing (Table 35.5). Phenotypes that are not known to include features of CP are marked with an asterisk

Gene	Gene listed in Table 35.4	Mendelian	OMIM number	Phenotype	Patients
AKT3	***N***	AD	615937	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2*	Matthews 16
ALS2	***N***	AR	607225	Spastic paralysis, infantile onset ascending	Srivastava 2
AMPD2	***N***	AR	615809	Pontocerebellar hypoplasia, type 9	Takezawa 11, Jin F033–003
AP4B1	***Y***	AR	614066	Spastic paraplegia 47, autosomal recessive	Rosello 1
AP4M1	***Y***	AR	612936	Spastic paraplegia 50, autosomal recessive	Jin F623–003
AP5Z1	***N***	AR	613647	Spastic paraplegia 48, autosomal recessive	Jin F342–003
ASXL1	***N***	AD	605039	Bohring-Opitz syndrome*	Matthews 10
ATL1	***Y***	AD	182600	Spastic paraplegia 3A, autosomal dominant	Rosello 5, Rosello 11, Rosello 18, Jin F050–003
ATP1A3	***N***	AD	614820	Alternating hemiplegia of childhood 2	Matthews 21
CACNA1A	***N***	AD	617106	Developmental and epileptic encephalopathy 42	Takezawa 9
COL4A1	***Y***	AD	175780	Brain small vessel disease 1 with or without ocular anomalies	Van Eyk 204
CSTB	***N***	AR	254800	Epilepsy, progressive myoclonic 1A (Unverricht and Lundborg)	Matthews 2
CTNNB1	***N***	AD	615075	Neurodevelopmental disorder with spastic diplegia and visual defects	Takezawa 3, Jin F066–003
CYP2U1	***N***	AR	615030	Spastic paraplegia 56, autosomal recessive	Takezawa 5
DGUOK	***N***	AR	251880	Mitochondrial DNA depletion syndrome 3	Srivastava 3
EHMT1	***N***	AD	610253	Kleefstra syndrome 1*	Matthews 5
ELP2	***N***	AR	617270	Mental retardation, autosomal recessive 58	Srivastava 1
ERLIN2	***N***	AR	611225	Spastic paraplegia 18, autosomal recessive	Srivastava 64
FARS2	***Y***	AR	617046	Spastic paraplegia 77, autosomal recessive	Jin F629–003
GCDH	***Y***	AR	231670	Glutaricaciduria, type I	Matthews 4
GNAO1	***N***	AD	615473	Developmental and epileptic encephalopathy 17	Rosello 15, Takezawa 7, Matthews 1
GNB1	***N***	AD	616973	Mental retardation, autosomal dominant 42	Rosello 17
IFIH1	***N***	AD	615846	Aicardi-Goutieres syndrome 7	Rosello 2
ITPA	***N***	AR	616647	Developmental and epileptic encephalopathy 35	Matthews 18
ITPR1	***Y***	AD	117360	Spinocerebellar ataxia 29, congenital non-progressive	Schnekenburg 2
KCNC3	***Y***	AD	605259	Spinocerebellar ataxia 13	Schnekenburg 1
KCNJ6	***N***	AD	614098	Keppen-Lubinsky syndrome	Matthews 13
KCNQ2	***N***	AD	613720	Developmental and epileptic encephalopathy 7	Srivastava 50
KIDINS220	***N***	AD	617296	Spastic paraplegia, intellectual disability, nystagmus, and obesity	Matthews 22

(continued)

Table 35.6 (continued)

Gene	Gene listed in Table 35.4	Mendelian	OMIM number	Phenotype	Patients
KIF1A	***N***	AD	614255	NESCAV syndrome	Van Eyk 174, Van Eyk 781
L1CAM	***N***	XL	303350	MASA syndrome	Van Eyk 724
MECP2	***N***	XL	312750	Rett syndrome	Matthews 8
MECP2	***N***	XL	300055	Mental retardation, X-linked, syndromic 13	Matthews 14
NAA10	***N***	XL	300855	Ogden syndrome	Matthews 9
NT5C2	***N***	AR	613162	Spastic paraplegia 45, autosomal recessive	Van Eyk 718, Jin F444–003
PANK2	***N***	AR	234200	Neurodegeneration with brain iron accumulation 1	Srivastava 15
PGK1	***N***	XL	300653	Phosphoglycerate kinase 1 deficiency	Rosello 8
PLP1	***Y***	XL	312080	Pelizaeus-Merzbacher disease	Matthews 3
RNASEH2B	***Y***	AR	610181	Aicardi-Goutieres syndrome 2	Rosello 20
SCN2A	***N***	AD	613721	Developmental and epileptic encephalopathy 11	Takezawa 17
SCN3A	***N***	AD	617938	Developmental and epileptic encephalopathy 62	Matthews 28
SPAST	***N***	AD	182601	Spastic paraplegia 4, autosomal dominant	Rosello 4, Takezawa 6, Takezawa 10, Srivastava 44, Matthews 20, Jin F082–003
SPATA5	***N***	AR	616577	Epilepsy, hearing loss, and mental retardation syndrome	Rosello 14
SPG11	***N***	AR	604360	Spastic paraplegia 11, autosomal recessive	Jin 84084P
SPTBN2	***Y***	AD	600224	Spinocerebellar ataxia 5	Schnekenburg 4
ST3GAL5	***N***	AR	609056	Salt and pepper developmental regression syndrome	Srivastava 54
STXBP1	***N***	AD	612164	Developmental and epileptic encephalopathy 4	Takezawa 12, Srivastava 71
TBCK	***N***	AR	616900	Hypotonia, infantile, with psychomotor retardation and characteristic facies 3	Matthews 25
TCF4	***N***	AD	610954	Pitt-Hopkins syndrome	Matthews 11
TMEM67	***N***	AR	216360	COACH syndrome 1	Matthews 6
TUBA1A	***N***	AD	611603	Lissencephaly 3	Jin (Table 35.2)
TUBB4A	***N***	AD	612438	Leukodystrophy, hypomyelinating, 6	Matthews 15
UBE3A	***Y***	AD	105830	Angelman syndrome	Srivastava 51
WDR45	***N***	XL	300894	Neurodegeneration with brain iron accumulation 5	Matthews 7
ZBTB18	***N***	AD	612337	Mental retardation, autosomal dominant 22*	Srivastava 8

genetic conditions that may present as CP (Table 35.4). The clinical features of most of these conditions in Table 35.6 are known to overlap with those of CP, but this is not true for a few of them (marked with an asterisk in Table 35.6). Whether the observation of apparently disease-causing variants of these genetic loci among patients with CP or atypical CP represents an expansion of our knowledge about the phenotypic spectrum of these rare genetic diseases or is simply coincidental is currently uncertain. It is noteworthy, however, that all of the genetic conditions in which a CP-like phenotype occurs also include other neurological abnormalities, and often non-neurological anomalies as well (Tables 35.4 and 35.6). Jin et al. [63] also demonstrated substantial overlap among the genes associated with CP and those associated with intellectual disability, autism or epilepsy.

Epigenetic Studies

Epigenetic mechanisms regulate the transfer of information from the genome, allowing different cell types, organs, body systems and the individual to develop from an undifferentiated zygote and to function throughout life. Although this concept is easy to understand, defining epigenetics in a precise scientific fashion has been surprisingly controversial [68]. Key aspects of epigenetic mechanisms are their dependence on features of the chromatin outside of the DNA sequence itself and the stable, but not invariably fixed, transmission of the epigenetic state of a cell through mitosis and over time. Epigenetic mechanisms also provide a means by which the environment can influence genomic function [69].

Laboratory animal studies have clearly established the importance of epigenetic mechanisms in neurodevelopment and adult neurological function, and many observational investigations are consistent with similar roles in humans [70]. The best-studied epigenetic systems are methylation of DNA and acetylation of histone proteins, but other covalent DNA or histone modifications, non-coding RNAs, and four-dimensional alterations of chromatin structure and its relationship to the nuclear membrane may also act in epigenetic regulation. Moreover, epigenetic changes of one kind can affect other kinds of epigenetic alterations in a multidimensional regulatory network [70].

Crowgey and her associates [71] performed genome sequencing of white blood cell DNA from 16 adolescents with spastic CP and 16 control subjects. Sequencing reads from 1.5 million CpG methylation sites throughout the genome were selected bioinformatically, and the degree of methylation at each site was quantified. Comparison of the CP and control groups found significantly increased or decreased methylation at 0.4% of the CpG sites assessed. Because the study was performed in adolescents, it was not possible to determine whether the methylation differences found reflected the presence of spastic CP (or its treatment) or were markers of the processes that caused the CP in these patients.

This issue was not a concern in a study performed on DNA obtained from archived newborn blood spots of 23 children with various forms of CP and 21 unaffected controls [72]. Using a standard microarray assay of 450,000 variably methylated genomic loci, this study found significantly different methylation of 0.05% of the loci tested. The authors suggest that differential methylation at these loci might predict the development of CP in a child, but, given the probable aetiological heterogeneity of the patients studied, it is unlikely that these differences provide any insight into underlying genetic factors.

Monozygotic twins, who are identical genetically but are discordant with respect to CP, provide an opportunity to assess the effect of non-genetic factors on methylation patterns. Mohandas and her colleagues [73] used a standard 450,000 locus methylation microarray to test archived newborn blood spots from 15 monozygotic pairs in which one twin developed CP and the other did not. No probes were found that exhibited statistically significant differential methylation between the twins with CP and the unaffected co-twins after adjusting for multiple testing, but top-ranked differentially methylated probes below the statistical cutoff involved genes that were associated with immunity and inflammation or with epileptic encephalopathy.

The findings were different in a study of four pairs of monozygotic twins who were discordant for CP and in whom genome-wide methylation was assayed by reduced representation bisulphite genome sequencing [74]. One hundred ninety differentially methylated genes were identified among the discordant twins. Enrichment analysis showed associations with genes involved in cerebral atrophy, and pathway analysis suggested involvement in the biosynthesis of antibiotics, glycolysis/gluconeogenesis and propanoate metabolism.

The Genetics and Genomics of Cerebral Palsy

Building on earlier family and twin studies, recent genomic investigations have clearly demonstrated that genetic factors of major effect cause CP in many patients. Most CNVs and small alterations of nucleotide sequence that have been found to cause CP or atypical CP arise as a result of de novo mutations, so studies that depend on the recurrence frequency within families substantially underestimate genetic contributions to the aetiology of CP.

Studies of series of patients with typical CP suggest that at least 4% have disease-causing CNVs [75–77] (Table 35.3) and at least 14% have disease-causing SNVs or indels [63] (Table 35.5). The rates of disease-causing genomic lesions are substantially higher among patients with atypical CP (Tables 35.3 and 35.5). Mutations of many different genetic loci can produce a CP-like phenotype (Tables 35.2, 35.3, 35.4, and 35.6). It seems likely that additional major genetic causes of CP will be recognized as more patients are tested, more sensitive tests (e.g. sequencing of the entire genome) are used, and bioinformatics and clinical interpretation of genomic data improve.

The importance of genetic variants of minor effect and of epigenetic modifications in producing a multifactorial predisposition to CP is less clear. These factors are likely to exist on theoretical grounds, but their involvement has been difficult to demonstrate convincingly. This is probably because of the variety and complexity of such multifactorial predispositions and of the interactions among them in different combinations.

Recognizing the specific cause of CP in a patient is essential to providing optimal clinical management for each affected individual. The financial, emotional and social costs for patients and families affected with CP are great, and obtaining a precise diagnosis provides families an ‘enhanced compass’ that improves overall well-being [78, 79]. Recognizing a specific genetic cause may also facilitate access to educational and social services beyond those that are related to the patient’s physical disability. In addition, treatment targeting pathophysiology is available for a subset of atypical CPs, namely those caused by inherited metabolic diseases [80]. Examples include congenital neuro-transmitter defects and inherited disorders of amino acid metabolism. Early recognition and initiation of therapy (e.g. medical diet, vitamin supplementation, liver transplantation or medica-

tion) is essential before irreversible damage is done in patients suffering a treatable Mendelian inherited metabolic disease. Time is brain!

Patients who receive genetic diagnoses and their families benefit by obtaining knowledge of the cause and projected natural history of their condition, and a precise genetic diagnosis is essential for accurate genetic counselling about recurrence in a family. Finally, obtaining a genetic diagnosis ends an expensive, time-consuming and emotionally draining 'diagnostic odyssey' for many families.

In a substantial fraction of patients with CP, and especially in those whose CP is atypical, an underlying genetic disease is responsible for the neuro-developmental abnormalities. Trio exome sequencing and chromosomal microarray analysis or trio genome sequencing with bioinformatics analysis for CNVs as well as SNVs and indels are clinically indicated in the initial workup of CP patients.

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Multiple Choice Questions

1. The mode of inheritance in the majority of cerebral palsy patients is:
   (a) X-linked dominant (de novo)
   (b) Autosomal recessive
   (c) **Autosomal dominant (de novo)**
   (d) None of the above
2. Establishing a diagnosis in cerebral palsy has implications for
   (a) Supportive care
   (b) Prognosis and counselling
   (c) Prevention and treatment
   (d) **All of the above**
3. In patients with cerebral palsy, genetic aberrations occur with the following frequencies
   (a) **disease-causing copy number variants: 4%, and single nucleotide variants or indels: 14%**
   (b) disease-causing copy number variants: 4% and epigenetic signatures: 21%
   (c) single nucleotide variants or indels: 14% and epigenetic signatures: 21%
   (d) structural and numeric chromosomal abnormalities: 13% and single nucleotide variants or indels: 14%
4. The yield of genetic/genomic testing increases if the following features are present:
   (a) positive family history for cerebral palsy, periventricular leukomalacia on neuro-imaging, progressive disease course
   (b) **progressive disease course, multi-organ involvement, affected siblings**
   (c) unexplained death in the family, progressive disease course, normal neuro-imaging
   (d) abnormalities on prenatal sonogram, normal newborn screening, behavioural problems

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Pre-Clinical Models of Cerebral Palsy

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Learning Objectives

- Identify and explain factors to consider when choosing an animal model for CP research
- Understanding the timeline of brain development in animal models, to be able to extract research data for the human
- Understand the structure and function of animal model placentas, to be able to extract research data for the human
- Identify methods to induce CP in an animal model
- Determine which animal model to use based on criteria/feasibility of research

Highlights

- Factors to consider when choosing an animal model for CP research: timeline of brain development (precautial like humans vs. non-precautial), placental structure and function (i.e. humans have...), cost of purchasing and housing the animal, size of the animal, and number of births (singleton vs. multiple). Each factor has it's pros and cons and they all must be taken into consideration before choosing an animal model.
- Timeline of brain development differs based on the animal; development can be precautial or non-precautial. Precautial refers to the majority of brain development occurring prior to birth while non-precautial refers to the majority of brain development still taking place after birth.
- Placental structure and function is different between animals; some animals have a similar placental structure to humans (discoid), but may lack functional similarity, while others will have a different structure with a similar functionality
- Most common methods to induce CP in an animal model for research are hypoxia-ischemia (usually via artery ligation and hypoxic air exposure) and intrauterine inflammation via lipopolysaccharide endotoxin exposure

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Introduction

Cerebral Palsy Definition and Phenotypes

Pre-clinical animal models of cerebral palsy (CP) are important for the understanding and translation of underlying mechanisms, treatment determination, and the prevention of CP to the clinic [1]. Models of all types should be representative of the CP phenotype in several different ways: (1) etiology/ies of CP, (2) underlying mechanisms at the cellular and molecular level, (3) the known underlying anatomic and pathologic underpinnings of CP, and (4) The physical and behavioral outcomes. CP is characterized as a group of permanent disorders that affect movement and posture, and limit activity, which is due to non-progressive disturbances of the infant or fetal brain [2]. It is also characterized by the

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presence of frequent co-morbidities in upwards of 45% of children, inclusive if sensory, cognitive, and mental health disorders. It has most frequently been affiliated with damage of white matter tracts of the perinatal brain, largely in the periventricular region [1, 3]. Damage to these areas largely occurs in the premature infant of 26–34 weeks gestation, but certainly it has been identified in the late preterm and term infant. Diagnosis of the disorder is typically in the first year of life through observation of clinical symptoms, historical features, and neurological signs [2, 4]. CP is most often characterized as being spastic, dyskinetic, ataxic, hemiplegic, and less commonly hypotonia. Spasticity is described as the resistance of a muscle to stretch and is present in over 80% of CP cases. Spastic CP can be further classified into unilateral-hemiplegic, bilateral-quadriplegic, or diplegic involving the lower extremities only [2]. Dyskinesis is the improper control of voluntary motor function usually associated with damage to the motor cortex, basal ganglia, thalamus, cerebellum, and/or corticospinal tract [2, 5]. An increase in muscle stretch reflex is denoted as hyper-reflexia, where children often have fixed flexed configurations of extremities [2, 5]. Hypotonia is a decrease in muscle tone and is commonly a result of dysfunction of the peripheral nervous system but can also occur due to damage of the central nervous system [2].

Considerations of Animal Models

Parturition across species varies depending on the precocial or non-precocial development of the fetus, as well as differences in the duration of gestation (Fig. 36.1) [6]. Non-human primates remain most closely related to humans in terms of pregnancy due to their long gestation period and precocial offspring, compared to other animal models [7]. Guinea pigs, rabbits, and sheep also deliver precocial young. Guinea pigs and sheep have a longer gestational period and deliver small litter sizes, similar to humans, while other murid rodents deliver non-precocial, larger litter sizes, and much development occurs post-natally [8]. The sheep completes much of their development prenatally, while rabbits, guinea pigs, and humans have ongoing perinatal development [9, 10]. Murid rodents (mice and rats) are popular as a preclinical animal model for pregnancy trajectories as they are

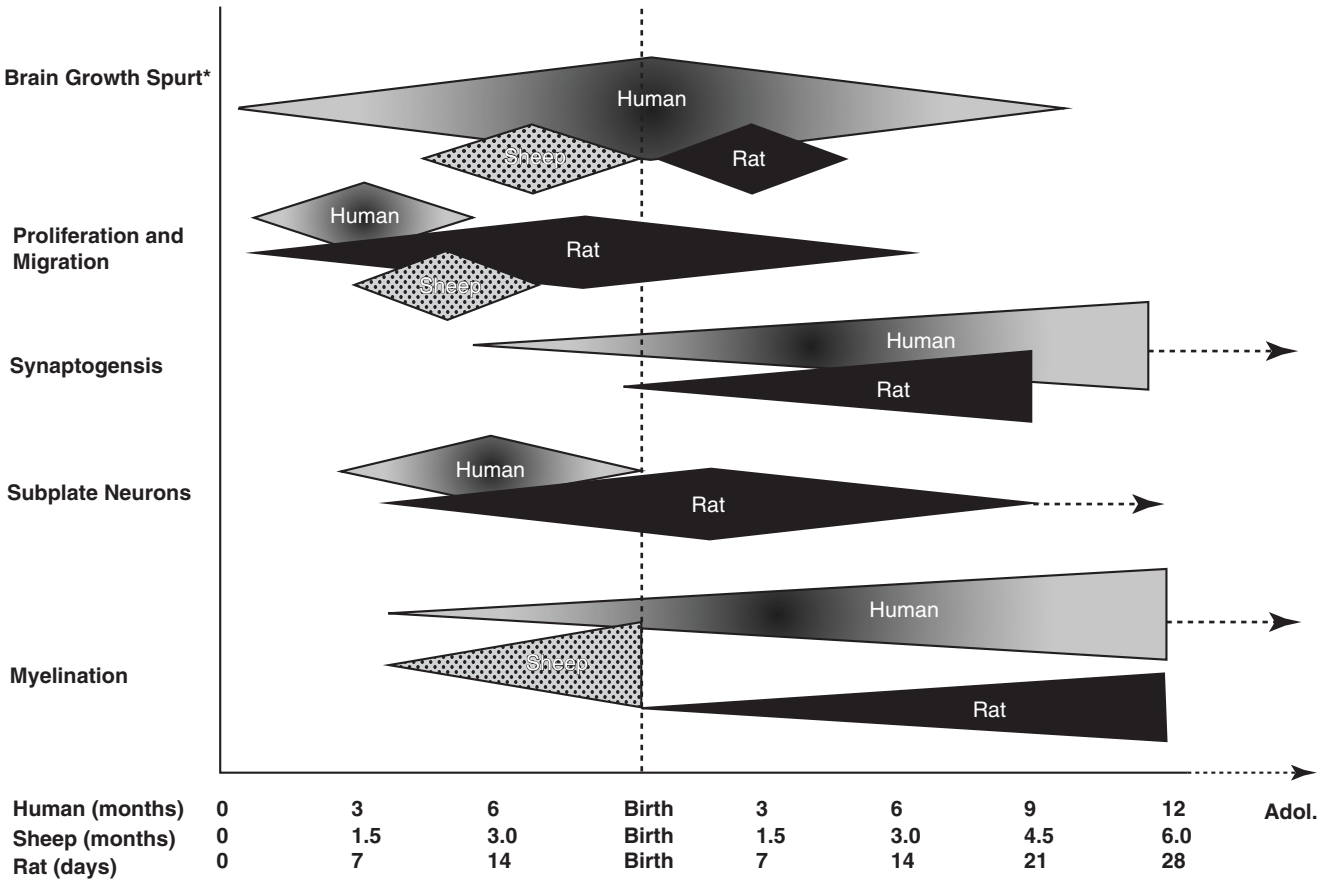

Fig. 36.1 Comparison of several parameters of brain development across species. Note the difference in duration, with timing being in months for the human and sheep and days for the rat. *Brain Growth Spurt adapted from Dobbing

small, affordable, easy to house, have a short gestational period therefore making studies feasible in terms of time, and are much easier to house and maintain for chronic behavioral outcome studies [7, 8]. Cost and ethics are also considered in animal models. Usually, the larger and/or more human-like the animal is, the greater the challenge to ethics approval, and the greater the cost [8].

The placenta is the lifeline of the mother to the fetus. It performs functions vital to the survival of the pregnancy and should be taken into account when considering pre-clinical models of perinatal brain injury. In other words, in spite of the insult to the fetus in the experimental paradigm, what contribution is the placenta making, either positive or negative. The placenta is classified based on gross shape and trophoblast organization between chorion and uterine wall [11]. Across all species the placenta functions to transfer nutrients from the mother to fetus, but the structure of the placenta and specificity how it functions does differ across species [7]. Primates, murid rodents, and rabbits all share a discoid or bi-discoid, hemochorial placental morphology [11]. Although the structure is similar, gene regulation in relation to pregnancy is quite different in murid rodents compared to humans [8]. The guinea pig and piglet model have a different placental structure, which is epitheliochorial and diffuse. Unique to the guinea pig and human is the high and increasing concentration of progesterone during parturition which is important during human pregnancy. The sheep has a cotyledonary and epitheliochorial placenta, again quite different from the human, but gaseous exchange and placental vasculature tend to be similar; however, they are of a completely different superorder than rodents and primates [7, 8]. In most models of perinatal brain injury, placental function is not taken into account. Indeed, all too often, obstetrical, neonatal, and pediatric research endeavors are approached separately. An area in which greater networking and collaborative opportunities abound.

Ideal Animal Model

Factors to be considered when creating an animal model for CP include cost, size, number of births, placental structure and function, timeline of brain maturation, and outcomes representative of CP and developmental disability. An ideal animal model would be one that is feasible in cost, and this would consider not just the cost of purchasing and housing the animal but also the cost of staff with certain expertise and equipment that may be required to induce CP. The size of the animal would also be under the umbrella for cost as the smaller the animal would generally mean the lower the cost. Ideally, a researcher would want an animal that will most represent the human condition; humans commonly have singleton births; however, multiple births would also be advantageous as more than one infant or fetus can be studied at each pregnancy. In addition, a placenta structure that is similar to a human would be preferential, or a placental structure that is different, but functions similar to that of a human. As the human brain develops perinatally, an ideal animal model would be one that also has perinatal brain development, similar to the human. The ideal model would be able to represent CP acutely following injury, in order to determine the underlying biophysiologic, biochemical, genetic, and epigenetic features, resulting in long-term brain damage and outcome. Perhaps most importantly, the model will have the capability of long-term survival, given that it is this behavioral and neuropathology aspects, which are now recognized as the gold-standard in the development and testing of new therapeutic interventions, and can enable the long-term effectiveness of the model and potential therapies.

Pre-Clinical Animal Models

Murid Rodents

Murid rodents, mice and rats, are a common animal used to understand the physiology of pregnancy trajectories and developmental outcomes. Protocols including hypoxia-ichemia (HI) and infection have been used in murid rodents to induce fetal brain injury and thereby, phenotypes of CP. Rice-Vannucci was the first to describe that neonatal model of HI, as an adaptation of the adult Levine model of stroke in the adult rodent. For the last 30 years, this model has been the most popular in studying perinatal brain injury, though adaptations of this model in rodents and mice have also been developed [6]. Originally performed in 7-day-old Wistar rats, under anesthesia, the left or right common carotid artery of each pup is exposed and ligated. The ligation can be permanent in nature or temporary, though it is usually permanent. Pups were returned to their mom for 2–3 h. Control pups underwent the surgery without ligating the artery. All pups were then placed in airtight jars (hypoxic) and exposed to humidified nitrogen-oxygen mix (92:8%); jars were partially submerged in 36.5 ± 0.5 °C water bath, to maintain an optimal thermal environment [12]. This model induces brain injury after birth, at postnatal day 7. That age was determined originally to be a late pre-term or term equivalent age in the human. Subsequent studies, however, argue that a more appropriate rodent age to equal that of humans at birth (40 weeks gestation) is between 10 and 12 days of age. As has been shown earlier in this chapter, aspects of brain development vary among species. At 7 days of age, their begins to be the onset of significant oligodendrocyte maturation in the murid rodent [12]. This persists through the first weeks of life, and, as in the human, takes some time to complete. Nonetheless, the Rice-Vannucci

model continues to be used in multiple therapeutic studies for CP, including human cord blood cell transplant [13], baclofen as a neurotransmitter inhibitor [14], mammalian/mechanistic target of rapamycin (mTOR) pathway inhibition [15], maternal low protein diet [16], and magnesium sulfate treatment, and of course hypothermia, and a host of other mechanistic and therapeutic studies [17].

Intrauterine inflammation is associated with an increased risk of periventricular leukomalacia (PVL), or perinatal white matter damage [6]. Bell et al. created an inflammatory animal model leading to CP using Fischer 344 and Lewis rats. Pregnant dams at embryonic day 15 were anesthetized (4% halothane), and given a dose of lipopolysaccharide (LPS) endotoxin (0.1 mg/kg) intracervically based on maternal weight. On day 20 of gestation, a hysterotomy was performed, pups were weighed, and brains were fixed for pathology which led to findings of increased brain cell death and an increase in pro-inflammatory cytokines [3, 18]. Another study using this same model but with 0.15 mg/kg LPS found a deficit in motor and sensory development in neonates. They found a delay in eye opening and in cliff aversion, as well as a decrease in staining of mature oligodendrocytes compared to controls, showing white matter injury [19]. In a similar experiment by Robertson et al., pups were given subcutaneous injections of LPS due to the presence of oligodendrocytes in the rat during this time frame. On postnatal days 2 to 6, pups were given increasing doses of LPS from 30 to 120 μg/kg to prevent tolerance. This study found that LPS exposure induced hyperactivity in the pups, but not necessarily CP-associated phenotypes [20]. In all or a majority of the LPS studies, a commonality is that CP phenotypes represented are minor and only present early on after birth, and they do not persist into adulthood. This LPS model has been used in studying CP therapeutics such as environmental enrichment [21] and erythropoietin and melatonin treatment [22].

Strata et al. used a Sprague Dawley rat model to investigate the organization of the primary motor cortex, M1, and effects of perinatal asphyxia. There were three experimental groups in this study design: perinatal asphyxia, or sensorimotor restriction, or both. In the perinatal asphyxia groups, pups on the day of birth and the day after birth were placed in a 100% $N_{2(g)}$ chamber for 12 min, and then release and resuscitated. In the sensorimotor restriction groups, pups were restrained by bounding extended hindlimbs together for 16 h a day between days 2 and 28 after birth [23]. The sensorimotor groups (with and without asphyxia) showed a much slower growth rate and lower overall weight, high Ashworth scores of resistance, degraded motor performance through open field test, lower score of suspended bar, and shorter time for rotor rod testing compared to asphyxia alone and control pups [23]. Although this may be an effective model for disuse in CP, it is not the most representative of CP as the injury in this model is not direct; it is a conditioning which occurs over many days after birth. Another study performed modified this same protocol by investigating effects of perinatal asphyxia, sensorimotor restriction, and LPS induction at gestational day 17; they discovered that LPS sensitizes the brain, making it more susceptible to HI environments [24].

A study done by Girard et al. combined both the HI and inflammation intervention to create a model that better investigated the double-hit theory of perinatal brain damage [25, 26]. They first induced an inflammatory response via infection of LPS at gestational day 17. Following birth on postnatal day 1, pups underwent a unilateral right common carotid artery ligation, as in the Rice-Vannucci model [12, 25, 26]. They found cerebral lesions more severe than LPS or HI alone, but were more representative of what has been found in human CP. They also found a significant decrease in spontaneous motor function and altered coordination, and cognitive impairment compared to LPS or HI alone, and again better representative of CP [25–27].

Placental Insufficiency

Intrauterine growth restriction caused by unilateral or bilateral uterine artery ligation was first introduced by Wigglesworth et al. [1, 2] Their findings were a combination of fetal growth restriction (FGR) and periventricular leukomalacia. Others have utilized this model to investigate the metabolic alterations of FGR with the development of determinants of adult disease. [3–6] Yager et al. [7–9] have utilized a model of bilateral uterine artery ligation in E20 gestation Long-Evans rats. Spontaneous vaginal delivery resulted in findings of white matter injury which mimic those of periventricular leukomalacia in the premature infant. Moreover, short-term behavioral outcomes reflected a delay in development [9]. Longer term outcomes indicated that these delays were similar to cognitive, attentional, and motor abnormalities that one would see in those with developmental disabilities (unpublished data). These findings were replicated in a model of intrauterine LPS intraperitoneal injection in E19 and E20 rats, every 12 hours for 4 doses. Once again, the newly born rat pups displayed evidence of developmental delay, though there were very few neuropathologic alterations; findings that, once again parallel those of some congenital forms of delay, such as autism and attention deficit hyperactivity disorder [8]. This model, along with that produced by others [10–13], [14, 15] provides the potential for a preventive approach to brain injury in the newborn. Remembering that previous studies have indicated that the majority of injury begins prior to the time of birth, and only between 10 and 20% occurs at the time of birth during labor and delivery, intrauterine models of perinatal brain injury hold great potential for developing preventive therapies.

Guinea Pig

Guinea pigs are a good model for pregnancy as they have many factors that parallel the human condition. They have a placenta with comparable composition giving rise to similar upregulation/downregulation of gene products. They also undergo a significant portion of brain development prior to birth; in utero, the brain weight reaches over 80% of the adult brain, unlike other animals, and they have a long gestation, similar to humans [28, 29]. Kim et al. created an HI model of the guinea pig. They used time-mated pregnant Hartley-Duncan guinea pigs, and induced a hypoxic environment in a plexiglass chamber for 14 days with only 10.5% O_2. At 13–21 days before birth (46–49 days of gestation, 70% of gestation), chronic fetal hypoxia was induced [28, 30]. Time-mated pregnant Hartley-Duncan guinea pigs were used. At 70% of gestation, they induced a chronic hypoxic environment in a plexiglass chamber for 14 days with only 10.5% O_2. Newborn guinea pigs were used in a longitudinal study including non-invasive diffusion tensor imaging, T2 mapping, and T2-weighted magnetic resonance imaging techniques, up to 6 weeks of age, and for pathologic purposes to determine HI-induced neuronal injury and loss. They found hippocampal volumes in HI offspring to be significantly smaller than the normoxic control group, which was agreeable with significant neuronal loss and reactive gliosis in guinea pig neonates; and these findings are consistent with commonalities of CP [28, 30].

Rabbit

Global HI has been a popular method to induce characteristics of CP in the rabbit animal model. Derrick et al. created a model of global hypoxia in the rabbit via uterine ischemia. They used New Zealand white rabbits and monitored timed pregnancies. At 70% gestation (E21 or E22), dams were anesthetized with intravenous fentanyl (75 mg/kg/h) and droperidol (3.75 mg/kg/h); doses were later dropped by 60% to allow spontaneous breathing through a mask which maintained normal arterial pH and pressure. Spinal anesthesia at L4-L5 vertebrae was administered with 0.75% bupivacaine. A 4F Fogarty arterial embolectomy catheter was introduced into the left femoral artery and balloon was inflated with 300 mL of saline. Blood pressure was measured at right lower extremity to confirm continuous ischemia (30–40 min). Following the procedure, the balloon was deflated and the catheter was removed. Dams underwent hysterotomy 24 h after the procedure (preterm, 23 days gestation) or delivered at 31 days gestation (term) [9]. Following the HI procedure and delivery, behavioral testing was completed including locomotion, tone of forelimbs and hindlimbs, righting reflex, and suck and swallow. Researchers found that the rabbit model was beneficial in testing motor abnormalities which were spontaneous and easily measurable compared to rodents [9, 31]. They found HI kits to have difficulty in motor function including head turning in response to olfactory stimuli; oral motor stimulation response also showed difficulty in suck and swallow [9]. A later study also found that sensory and motor deficits were associated with damage to olfactory neurons [32, 33]. Neurobehavior was further tested in this model using swim tests to establish its consistency with CP [34]. This model represented a rare phenotype of CP in some kits, which was hypertonia, likely due to localization of white matter injury which they found to be in the thalamus and basal ganglia [9, 31]. Although popular, the rodent model of HI-induced CP has a fault due to their high levels of xanthine oxidase which is important in free radical scavenging, while the rabbit and human both have low levels of this enzyme [10, 35]. This model also produced consistent unchanging hypertonia which is pathognomonic of CP. There was no significant difference in how HI affected sex; however, female kits were more likely to survive, but prone to be hypertonic, compared to males [35]. Overall, the rabbit HI shows several phenotypes of human CP including motor deficits (hypertonia and rigid posture), white and gray matter brain and spinal cord injury, and abnormal muscle fiber properties [4, 36]. This model has been used in researching therapeutics of CP including testing nitric oxide synthase inhibitors [37–39] and human umbilical cord blood cell therapy [40].

In addition to HI, infection is also a common method of inducing CP in animal models [4]. The difference between HI and infection, in relation to type of injury, is that HI primarily injures oligodendrocytes and neuronal cells, and secondarily is microglia activation, while infection has the reversed ordered [41]. Kannan et al. developed an animal model of maternal intrauterine infection and CP, as chorioamnionitis is linked to CP outcomes [42–44]. They also used New Zealand white rabbits with timed pregnancies, and injected *Escherichia coli* lipopolysaccharide (20–40 mg/kg) along the uterine wall at 28 days gestation. Kits were born spontaneously at 31 days gestation (term) [42, 43]. Myelination in humans and rabbits is similar as it begins in the third trimester and continues after birth. Endotoxin administration resulted in a rise of pro-inflammatory cytokines (interleukin-1β [IL-1β], IL-6, and tumor necrosis factor-alpha) and in immature oligodendrocytes, thereby reducing myelination and maturity [41, 42]. Further research also showed that endotoxin exposure resulted in decreased serotonin levels along with afferent neurons in the somatosensory cortex, which may lead to abnormal resulting in altered sensory processing and sensorimotor integration [45]. Behavioral tests of this animal model confirmed that LPS induces similar phenotypes to CP [9, 41]. The LPS

model of infection has also most prominently been used in studying a therapy for CP including polyamidoamine dendrimers for drug delivery [46–53].

Cat

Perinatal damage to the developing corticospinal tract (CST) represents motor deficits similar to those seen in CP. The CST is involved in motor control pathway for skilled movements, and during fetal development, there is a period of vulnerability when damage to the CST can result in long-term consequences [54]. Using inhibitory neurotransmitter, $GABA_A$ (γ-aminobutyric acid A receptor), in the motor cortex, which is the origin of CST in cats, Martin et al. were able to block CST activity in postnatal day 5 cats which resulted in a pattern of CST connections resembling hemiplegic CP. CST in cats and its activity dependence resembles that of humans, and aberrant CST produces motor circuit changes that are similar to those see in hemiplegic CP: elimination of strong contralateral projections from affected side, and the ipsilateral CST from unaffected side maintained connections to elicit motor responses [54]. This study found that cats with unilateral cortical inactivation developed impairments in reaching and visual guidance adaptive locomotion, compared to healthy control cats. For example, for the former, the target was too far to be grasped, and for the later, paws must have been seen and placed to avoid slipping. It is important to note that in this study, there was no actual damage to the brains of the cats; it was only the internal signals between the CST and the cerebellar system which was dysfunctional [54].

Pig

Unilateral lesioning of the brain produce abnormal neuron connections which cannot be done using other methods such as HI or infection. Common lesions of CP are ulegyria and subcortical damage, and with these lesions, a neurophysiological behavior similar to human pathology is expected in pigs, and would not require a large amount of technical staff and resources that HI or infection model would require [55]. Therefore, it may be less expensive as it does not require a complicated procedure for operative care. Andreani et al. performed a study in young pigs that elicited abnormal propagating electromyographic (EMG) polysynaptic current spreading surrounding the L4 nerve, to create upper motor lesions which are not present in the normal central nervous system of pigs or humans. Pig and human conduction speeds are similar and therefore the pig is a good model for clinical conditions [55]. They used young pigs of 30–40 kg in weight. After premedicating the animals with promethazine (20 mg/kg) by intravenous perfusion, they placed a venous catheter to continually monitor intraoperative electrocardiographics. Pigs were placed under anesthesia with a short induction of ketamine clorhidrate (40 mg) and isoflurane (5%) by mask, followed by intravenous perfusion of ketamine hydrochloric acid (50 mg/kg/h) and fentanyl (10 μg/kg/h). Following anesthesia induction, animals were intubated and ventilated by mechanical respirators. Intraoperative antibiotic coverage was performed using cephalexin (1 g) prior to surgery. The surgery included opening the dura matter, and performing cortical and subcortical lesions. Using b-polar forceps, the cortical surface of sylvian gyrus and sulcus was coagulated, leading to a midline coronal sulcus lesion. Aspiration of coagulated tissue was continued by subcortical dissection, and using unipolar coagulation directed in deep gray matter, internal capsule lesion was created between caudate and putamen nuclei [55]. Andreani et al. showed that this model developed lesions which produced anatomic and pathologic damage corresponding to natural lesions. Following the surgery, pigs were subjected to neurophysiological measurements of motor-evoked potentials (MEPs) and abnormal EMG polysynaptic current spreading. Pigs with lesions showed delayed latencies of MEPs and abnormal EMG spreading on fourth lumbar root, contralateral to stoke side, compared to control non-operated animals [55]. This model employed sustained hypoxia, unlike most models of HI which are usually abrupt, such as placental insufficiency. It is a good model to investigate effect of electrical stimulations as well as neuromodulation in spasticity, therefore, a good model for spastic CP [55].

Sheep

The sheep is a large animal that is well established as a human pregnancy model due to its ability to mimic current human practices of drug delivery route and dosage, and their longer gestation period which is similar to that of humans [56]. Huang et al. performed a study to examine the effects of corticosteroid use on myelination of white matter tracts in the corpus callosum. To ensure the suitability of sheep as a model for studying effects of corticosteroids, they confirmed that fetal plasma measurements of corticosteroid, betamethasone, concentration in maximum values, and clearance rates were similar to those of humans. They used a Marino sheep breed as their animal of choice. In this study, female and male Marino ewes were mated, and the pregnant sheep were examined at 50 and 85 days gestation via obstetric ultrasound to confirm singleton pregnancies. They pregnant sheep were given medroxyprogesterone acetate on gestational day 100 to prevent preterm birth, and were allowed to deliver naturally at term. During the last third of pregnancy duration, ewes received an intramuscular injection of betamethasone

at 0.5 mg/kg on gestations days 104, 111, 118, and 124 [56]. Significantly delays in myelination were found in the offspring likely due to effects betamethasone. Corticosteroids regulate oligodendrocyte differentiation and myelination. The corpus callosum completes its myelination in humans at approximately 10 years of age, and therefore is vulnerable to insults, such as the effect of corticosteroids, for an extended period of time during child development. Delayed myelination of the corpus callosum is commonly found in children with neurodevelopmental disabilities, and specifically abnormal development of the corpus callosum is highly associated with CP [56].

Non-Human Primates

Dieni et al. performed a study with baboons, *papio species*, and conducted procedures with interventions similar to what a human infant would encounter. Baboon mothers received antenatal steroids and infants were cared for in neonatal intensive care. This model was unique in that it did not require induced infection or other prenatal compromise such as growth restriction. They also showed that the baboon brain displays structural similarity to humans in regard to sequence of white and gray matter maturation [57]. Preterm baboons were delivered via hysterotomy at gestational day 125 ± 2, which is equivalent to 26–28 weeks gestation in humans, and were nursed to 140 days gestation, and then euthanized for neuropathology with sodium pentobarbitone (130 mg/kg). Dams received antenatal steroid therapy (6 mg betamethasone or dexamethasone) at 24 h and 48 h prior to delivery, and surfactant replacement was administered to neonates immediately after delivery. Blood pressure and gases were monitored by an arterial line placed into the descending aorta of the umbilical cord. Administration of drugs and fluid to the neonates was done via a venous line from the saphenous vein to the inferior vena cava. Infants were ventilated with a time-cycled pressure regulated ventilator with a humidifier maintained at 37 °C. Amino acids were administered at 24 h (1.5 g/kg/day) and 48 h (3 g/kg/day). Hypotension of infants was managed with volume replacement, dobutamine, dopamine, and epinephrine as needed. Premature infant vitals were monitored until euthanized at gestational day 140 [57]. Injuries that resulted were common to CP; most prominently, the neuropathology showed white matter and hemorrhage injuries, compared to control term infants. White matter injury included small patches of reactive astrocytes to extensive damage such as cystic lesions, endothelial hypertrophy, and activated microglia; on average 1.24% of white matter was injured. Subarachnoid hemorrhage was also found with damaged centers surrounded by fibroblasts, macrophage activity, and thickened capillary walls. Hippocampal damage, cerebral cortical, and deep gray matter damage were also found [57]. Inder et al. performed this same protocol and also found the most common injury to be white matter and hemorrhage [57, 58]. However, the staff and equipment required, cost of animals, and high order ethical considerations were a significant disadvantage to the model [58].

A major form of cerebral white matter injury is periventricular leukomalacia (PVL) which is associated with the development of CP [59]. Okabayashi et al. studied cases of spontaneous PVL-like lesions in neonatal cynomolgus monkeys, *Macaca fascicularis*. Neuropathology of experimental monkey brains were compared to control brains at 0 days old. Case 1 was a neonatal female delivered by cesarean at 163 days gestation (term is 165 days). Vaginal hemorrhaging caused difficulties during delivery and resulted in the neonate not breathing for several minutes before being resuscitated. Limb paralysis was observed 3 days later and the monkey became further debilitated due to insufficient sucking of milk. The monkey was treated with subcutaneous infusion of 5% glucose and emergency medical care, but later died naturally at 21 days after birth [59]. Case 2 was a neonatal male born 16 days prior to term. Limb paralysis and insufficient sucking of milk began the day after birth, and abnormal breathing 3 days after birth. Monkey was treated with 5% glucose and antibiotics, but later died naturally at 7 days after birth [59]. Although case 1 was of normal gestational age, the monkey suffered from asphyxia resulting in oxygen deprivation likely due to drop in maternal blood pressure or reduced blood flow to infant brain. Asphyxia led to lesions in the brain, markedly wide spread cavitation and loss of cellular elements in cerebral white matter similar to cystic PVL [59]. Case 2 was more prone to PVL as it is more common in preterm infants (26–34 weeks gestation in humans), due to immaturity of developing neurons, and presence of oxidative stress and free radical species which damage precursor oligodendrocytes. The preterm male also showed necrosis of cerebral white matter, astrogliosis in cerebral white matter and thalamus, decreased number of mature oligodendrocyte cells, and lower myelination, similar to non-cystic PVL [59]. In both cases, monkey weights were 290 g which is approximately 5–15% less than normal birth weight. Limbs and tails also had decreased range of motion; femoral muscle showed neurogenic atrophy due to damage of cerebral white matter. Tracheas also filled with a mixture of milk and mucus, and diffuse pulmonary lesions were found; likely due to insufficient sucking of milk [59].

McAdams et al. performed a model of acute perinatal asphyxia using umbilical cord occlusions (UCO) prior to delivery to produce moderate to severe hypoxic-ischemic encephalopathy (HIE) in pig tailed monkeys, *Macaca nemestrina* [60]. Mothers were placed under general anesthesia (sevoflurane), and infants were delivered 1–8 days prior to term (168 ± 2 days gestation) by hysterotomy, fol-

lowing UCO. Fifteen to twenty min of UCO, a duration of HIE comparable to human newborns, resulted in death or moderate to severe CP outcomes. Fetuses were stabilized by a team of neonatologists; resuscitation included endotracheal intubation, positive-pressure ventilation, chest compressions, and bolus epinephrine. APGAR scores were assigned at 1, 5, 10, and 20 min. Thermal support was initially provided by polyethylene sheets, radiant warmer, and heating pads, and then infants were placed in a thermal-neutral incubator [60]. Motor abnormalities consistent with CP were documented at 1 week, 1 month, and 8 months using the Ashford scale (0—normal to 4—affected) for rigidity in flexion/extension; animals were characterized as normal (no CP), mild CP, moderate CP, or severe CP/death [60]. McAdams et al. found that monkeys with CP showed a decrease in fractional anisotropy (FA) or multiple white matter tracts of the corpus callosum and internal capsule. Decreased FA has also been shown in human infants with HIE, and is associated with long-term outcomes. Decreased staining for cortical neurons, decreased cell density of cerebral white matter, and increased glial scarring of the brainstem were also present in animals with CP compared to animals without CP. There were no basal ganglia or thalamus injuries which are usually associated with dysfunctional neuromotor and cognitive outcomes; however, this is possible in dyskinetic CP, but less common in spastic CP [60]. This model developed CP in a third of experimental cases, and was more likely to show in males compared to females. It was also convenient as the non-human primate brain development is similar to humans in regard to neurocognitive testing which can be conducted overtime analogous to human testing; 9 months old monkey is equivalent to a 3 years old human. Difficulty in this model included the consistent duration of UCO varying from 15 to 18 min, and similarly to Dieni et al. and the baboon model, this was also costly because of the animal model chosen [57, 60].

Conclusion

Many factors go into choosing a pre-clinical model of pregnancy and delivery, and even further specificity for a disorder such as CP and the developmental disabilities. Larger animals such as non-human primates, sheep, or pigs, may be more representative of human births due to gestational time frame, placental anatomy, and ability to access the intrauterine cavity and umbilicus. However, they are costly, require maintenance, and are poor models for long-term recovery and behavior. Rodents, rabbits, and guinea pigs are smaller animals and are excellent for acute experimentation and understanding of mechanisms of injury, as well as long-term outcomes, behavioral analysis, and testing of therapeutic interventions. The smaller pre-clinical models require less maintenance, and are simply cheaper. Ethical considerations also come with all animal models, but in regard to primates, they are of a much higher order which can be more challenging. The placenta is another factor that contributes to a pregnancy model; although some placentas are structurally similar to humans such as the rodent placenta, others may be more functionally similar to humans such as the guinea pig. Development of the brain is also a significant consideration specifically for a CP model. The rodent model, although most popular due to low cost and reliability, develops much later after birth, postnatally, compared to the human brain which develops perinatally. Type of CP to be produced, such as spastic or hemiplegic, should influence the method of CP induction, whether it be HI, infection, manually creating lesions, or spontaneous preterm birth. Each pre-clinical animal model of CP has its advantages and disadvantages to account for such as staff expertise, equipment, and time; there is no one perfect or desirable choice. Considerations for the type of model to be considered must always focus on the "goal" of the study and questions to be answered.

Multiple Choice Questions

1. What factors should you consider when choosing an animal model for CP research?
   (a) Precautial vs. non-precautial development
   (b) Placental structure & function
   (c) Number of births
   (d) Size of animal
   (e) Cost
   (f) Measurable and representative outcomes of CP
   (g) **All of the above**
2. Which of the following animals have a non-precautial development?
   (a) Non-human primates
   (b) **Rats**
   (c) Guinea Pigs
   (d) Sheep
   (e) All of the above
3. Which of the following animals have 1. a similar placental structure and 2. a similar placental function, as humans?
   (a) **1. Rabbits, 2. Guinea Pigs**
   (b) 1. Rats, 2. Rabbits
   (c) 1. Guinea Pigs, 2. Pigs
   (d) 1. Pigs, 2. Rats

4. There are commonly two methods used to induce CP in an animal model, what are they?
   (a) Hypoxia-ischemia
   (b) Manually restricting muscle movement
   (c) Intrauterine inflammation via lipopolysaccharide endotoxin
   (d) Spinal damage to restrict muscle movement
   (e) **A & C**
   (f) A & B
   (g) B & D
5. Which animal model would you choose for the following criteria: large animal, precautial, singleton births
   (a) **Sheep**
   (b) Cat
   (c) Rat
   (d) Rabbit
6. Which animal model would you choose for the following criteria: low cost, small animal, multiple birth.
   (a) Sheep
   (b) Cat
   (c) **Rat**
   (d) Rabbit

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Therapeutic Approaches for the Treatment of Cerebral Palsy and Developmental Disability

37

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Learning Objectives

- Understand the various approaches of Therapeutic Approaches to Cerebral Palsy
- Recognize the availability of long term approaches to Treatment for CP
- Recognize the current accepted clinical standards of care regarding therapeutic hypothermia, and those in which pre-clinical trials have shown some benefit.

Highlights

- In utero CP prevention is vital to target the 85–90% of infants for whom too much damage has been done by the time of birth for rescue therapies to be beneficial.
- Of the various antenatal neuroprotectants that show promise in animal and clinical trials, magnesium sulfate ($MgSO_4$) and corticosteroids are considered in the current standard of practice.
- Therapeutic Hypothermia is the only currently accepted standard of care for perinatal hypoxic-ischemic brain injury
- Transcranial Magnetic Stimulation has recently been introduced in some centers to improve stroke and hemiparetic cerebral palsy outcome.
- Stem cell research for cerebral palsy has been implemented in several countries. It is not yet a standard of care in most developed countries. However, pre-clinical studies have been very encouraging.

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Section I: Prevention

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Introduction

Cerebral palsy (CP) encompasses a broad range of difficulties, predominantly with movement, but may include altered cognitive abilities, abnormal sensation, seizures, and mental health issues. CP is the most common motor impairment of childhood, with a global pooled prevalence of 2.0 to 3.5/1000 live births. This value is roughly 10 times higher in those born prematurely [1, 2]. The prevalence has not

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changed significantly over the past decade [3, 4]. However, there is evidence that this may be starting to decrease in some high-income regions, and in very preterm infants [4–7]. CP arises due to injury of the brain prior to or at the time of birth, or in some cases congenital abnormalities. The diversity of outcomes is largely explained by the type of injury, and the specific stage at which interference with the healthy course of development has occurred [4, 8–11]. It is thought that approximately 85–90% of the time, these initiating factors occur in utero during the fetal period, and the remaining 10–15% occur during birth or in infancy [4, 12–14]. However, the exact cause in any particular case is often not determined [15].

Although CP is permanent and non-progressive, the presentation may evolve as the child grows into their lesion and as varying degrees of accommodation and rehabilitation occur. CP is diagnosed clinically with a particular focus on motor function, which results in a diagnosis spanning several months or even years after the inciting factors had occurred [4].

Given that it has now been recognized that the inciting injury in the vast majority of infants occurs in utero, the following section will discuss approaches to the prevention of CP [16]. Particularly, we will focus on strategies to minimize these inciting factors for the development of CP from occurring in the first place, known as primary prevention. It should be noted that interventions to mitigate the damage in the 10–15% of infants who experience the inciting events during and after birth will be discussed in subsequent sections, the most successful of which has been the introduction of post-ischemic hypothermia [4, 17–24].

The focus of the following chapter, however, will be on human trials of the interventions aimed at the 85–90% of inciting events that occur in utero. Each section below will explore a different aspect of CP prevention, including (1) non preventable risk factors or those only preventable with pregnancy planning; (2) importance of maternal access to education, social supports and healthcare; (3) mechanisms of fetal brain injury; (4) prevention of premature birth; (5) prevention and treatment of antenatal infection; and finally (6) specific therapies for neuroprotection.

Non-Preventable Risk Factors/Pregnancy Planning

The pathophysiology of CP is complex and multifaceted; thus, there are numerous risk factors including male fetal sex, nulliparity or high parity, particularly long or short intervals between pregnancies, low or advanced maternal age, multiple gestations (therefore use of assistive reproductive technology), placental abnormalities, and maternal history of still birth, preterm birth, neonatal death, or multiple miscarriages [4, 25–32]. Although these factors are relevant to the development of CP, they are either non-preventable or only preventable with pregnancy planning. Additionally, genetic predispositions to CP are being unveiled increasingly in recent years, but at this point they are not yet sufficiently understood to serve as targets for prevention [33–37].

Education, Social Programs, and Consistent Healthcare Access

There are several factors related to maternal health and lifestyle that are known to confer risk for CP and do active harm to the developing fetal brain. Continued public health campaigns and education in clinical settings about how to avoid putting the fetal brain at greater risk are vital. Likewise, programs to support women who are having difficulties caring for themselves and the pregnancy, for example, due to financial constraints, substance use issues or intellectual disability also must continue to be available [29, 38]. Getting pre-existing mental and physical health conditions under control before becoming pregnant is ideal, but often not practical. Pregnant women with pre-existing conditions (e.g., epilepsy or seizures, thyroid conditions, hypertension, hyper- or hypothyroidism) or complications that arise throughout the pregnancy (e.g., pre-eclampsia, hypertension, placental conditions, abnormal fluid volumes) should have their health closely monitored throughout the pregnancy as these all have been associated with increased risk of CP [29, 39, 40]. Overall, decisions should be made with their healthcare provider to balance the risks of the uncontrolled condition with the risks of the therapies for both maternal health and fetal health in a way that aligns with the pregnant woman's values.

Inclusive Care Tip

Remember that pregnant patients are a diverse population, so it is important not to make assumptions. Not all people who become pregnant identify as women or consider themselves mothers. Use the pronouns and language that your patient prefers. Not all pregnant patients plan to continue the pregnancy. Ask the patient in a non-judgmental manner whether or not they plan to move forward with the pregnancy. If they plan to, or are undecided, proceed with appropriate prenatal care and counseling. If they do not plan to continue the pregnancy, it is inappropriate and insensitive to discuss fetal health.

Mechanisms of Fetal Brain Injury

Before discussing specific therapies that have been explored to prevent fetal brain injury, it is important to review mechanisms of fetal brain injury with respect to cerebral palsy. These mechanisms have complex interactions, not all of which are fully understood. Some of the known nodes on the

path to fetal brain injury are blood vessel immaturity, oxygen and glucose deprivation, oxidative damage, inflammation, cell excitotoxicity, apoptosis and necrosis, and myelination impairment. Here, we briefly review some of these mechanisms.

Premature infants are at particularly high risk of cerebral palsy. Partly due to their greater blood vessel immaturity compared to full-term infants makes them even more likely to experience intraventricular hemorrhage and periventricular leukomalacia (PVL) [13, 41]. PVL is due to poor blood vessel penetration in the periventricular and trigone regions between the 24th and 32nd week of gestation [42–44]. Thinly walled vessels penetrating and extending from the periventricular region attempt to meet with "end-arteries, penetrating from the surface of the brain. As they extend to reach each other to form a rich blood supply by term, gaps or watershed regions exist in the 'periventricular space." Interference with blood flow systemically can lead to this region being susceptible to ischemia and hence the predisposition to PVL.

With limited glucose, the synthesis of ATP, an essential energy molecule, is impaired. This results in inactivation of the Na+/K+ATPase, leading to cytotoxic cerebral edema and neuronal depolarization [45–47]. This can cause excessive buildup of glutamate and therefore, neurotoxicity which worsens neuronal swelling and disrupts membrane integrity, culminating in necrosis [47–50]. As well, the lack of ATP means that the energy needs of the fetus are not met. With inadequate oxygenation, the fetal brain which already has a higher oxygen demand than the adult brain suffers from ischemia and cell death, critically impairing normal bodily and cognitive growth and function [51]. Specifically, without oxygen present as a terminal electron acceptor, the electron transport chain is impaired and ATP production halted; instead, reactive oxygen and nitrogen species are formed [52]. Thus, even with reoxygenation and reperfusion, damage does not halt due to the presence of oxidative species [53].

Oxidative species, or free radicals, are highly reactive molecules that disturb the structures of relatively inert and important cellular components, including DNA, proteins, polysaccharides, and other building blocks in the human body [54]. Disruption to normal cellular functioning, such as maintaining the integrity of mitochondrial membranes and metabolic functioning, results in the release of proteins signaling programmed cell death [45, 55, 56]. This potential for oxidative damage is usually mitigated with scavenging molecules that sequester and neutralize oxidative species; however, in the fetal brain, these scavenging molecules are lower in quantity, placing the fetus at an elevated risk of oxidative damage [42–44, 51, 57]. Additionally, immature oligodendrocytes, which compose a large portion of the fetal brain, are particularly susceptible to free radicals and oxidative damage, resulting in brain white matter damage [51]. Hence, sequestering or inhibiting reactive oxygen species can provide long-term benefits of proper oligodendrocyte maturation, myelination, and white matter development [52].

With injury to the brain, exposure to a foreign antigen from a maternal infection, or other intrauterine infections, the fetus is at greater risk of prematurity [52]. Both the mother and fetus can mount an innate immune response, resulting in the recruitment of various white blood cells and the release of a vast array of chemokines, cytokines, and other proinflammatory molecules. Under normal circumstances, an immune response serves a protective function, but when it has been upregulated and skewed, bodily harm may result. For instance, with placental and fetal inflammation, the inflammatory cascade is upregulated in the fetal brain including the tryptophan–kynurenine pathway, which results in the production of neurotoxic metabolites [58]. With these mechanisms in mind, we will discuss some of the preventative measures that can target one or more of these pathways.

Prevention of Premature Birth

One of the growing risk factors for CP is premature birth, as it accounts for over half of CP cases and lasting neurological disabilities [59]. As such, tocolytics (medications used to prevent preterm birth) and other interventions with this aim have been investigated as a potential to prevent CP. However, tocolytic agents can delay delivery in the order of days, and it is unclear if this timeline is sufficient to reduce the incidence of CP [60–62]. For instance, when betamimetics were compared to placebo in a systematic review of 20 trials, they successfully delayed birth for several days, but not enough to significantly reduce preterm births <37 weeks. Only one trial reported CP outcome; this trial demonstrated slight favoring of betamimetic over placebo, but the values were not significant [60]. Indomethacin is a COX inhibitor that can be used as a tocolytic agent. Interestingly, in a study of this substance, infants exposed antenatally were born significantly earlier than those not exposed. CP outcome did not differ between groups [63, 64]. $MgSo_4$, discussed below for its use as a neuroprotectant, has also been explored as a tocolytic agent. However, it may have risks to bone health in both the pregnant woman and the fetus if used for too long [65]. Other research has deemed it ineffective for preventing preterm birth and found increased infant mortality [66]. Similarity, the MagNET trial found that higher doses of $MgSO_4$ were associated with increased combined pediatric mortality, Grade 3 Intraventricular Hemorrhage (IVH), PVL, and CP [67–69]. Progesterone appears more promising as it has been determined to be the most cost-effective prevention strategy for preterm birth (when paired with cervical length screening) [70–73]. A meta-analysis found progesterone to be effective in preventing preterm birth in women with previous

preterm births or women with short cervixes on ultrasound. In the former, it also improved several outcomes for the newborn, including a reduction in perinatal mortality. However, its effect on the outcome of CP was not reported [71]. However, one study explored neurological outcomes at 2 years of age and found reductions in fetal death and less evidence of brain injury on ultrasound in the progesterone supplemented group. Although they did not find improvements in standardized cognitive scores, it should be noted that these cognitive scores are a limited way of evaluating neurocognitive disability and may differ from CP outcomes, which again, were not assessed [74]. In terms of non-pharmacological interventions, there have been a few randomized controlled trials (RCTs) of cervical pessary to reduce preterm birth, but only one showed a significant reduction in preterm birth <34 weeks; once again, the authors did not explore the outcome of CP [75, 76]. Another intervention attempted for reduction in premature birth is exercise in obese women. Although exercise was associated with reduced preterm births, the CP outcome was unchanged [77].

Prevention of Infection

Half or more of preterm births occur in the context of infection [78]. Intrauterine infection is thought to be a prominent cause of CP [79–82]. Chorioamnionitis has also been shown by meta-analysis to have a relative risk for CP of 1.9, with an increased risk for CP of 140% with clinical evidence, and of 80% with histological evidence [83].

Thus, infection prevention and treatment have also been explored as a means of reducing preterm birth and therefore potentially CP. For instance, treatment of bacteriuria/ascending urinary tract infections (UTIs) and periodontal disease before delivery can make preterm birth less likely [78, 84, 85]. However, several studies have suggested that by the time infections are recognized and treated, it is "too little too late" to alter the physiological processes underway leading to preterm delivery, and/or fetal neurological damage [78, 83, 84, 86, 87]. Some antibiotics, such as erythromycin or co-amoxiclav, given during preterm delivery have even been shown to increase incidence of CP [88–90]. It is important that treatments and prevention strategies are fully assessed and their risks compared with the risk of the infection itself. Screening, earlier detection, infection prevention strategies as simple as frequent handwashing, and getting recommended vaccines before and during pregnancy [91] are likely well suited to benefit maternal and fetal outcomes [78, 86]. Antimicrobials must be used with caution [92] Further studies are needed to elucidate how to safely treat and prevent infections with the ultimate aim of CP prevention. As such, practice guidelines do not recommend the use of antibiotics to improve neonatal outcomes in cases of preterm labor and intact membranes [93].

Neuroprotection

Several therapies have been clinically studied for their role in neuroprotection. In various ways, the therapies below enhance the fetal ability to withstand and overcome neurological damage that could result in CP.

Magnesium Sulfate ($MgSO_4$)

Magnesium sulphate, an inorganic salt, may increase vasodilation and therefore potentially enhance reperfusion, have anti-inflammatory and antioxidant properties, and reduce excitotoxicity and cerebral metabolism [46, 48, 94–99]. $MgSO_4$ readily crosses the placenta and enters fetal circulation [46, 100, 101].

$MgSO_4$ has traditionally been used in pregnancy to treat high blood pressure and prevent pre-eclampsia [102]. In 1992, a study found that preeclamptic and non-preeclamptic mothers supplemented with magnesium sulfate had children with reduced risk of intraventricular hemorrhage (in both babies with a high and low risk of IVH) [103]. This was part of the impetus that shifted the focus of $MgSO_4$ research to explore it more broadly as a neuroprotectant. $MgSO_4$ has now been more heavily studied in humans than any other intervention for antenatal CP prevention. There have been a handful of large RCTs and meta-analyses. Most of these studies focus on those at imminent risk of premature delivery as this population is at greater risk of developing CP, therefore the trials are more likely to have discernable outcomes, and the risks of $MgSO_4$ exposure are more likely to be outweighed by the benefits. Few trials demonstrated significantly lower rates of CP in children of those exposed to $MgSO_4$ during pregnancy [104, 105]. Although most trials have lacked statistical significance, they have fairly consistently shown trends towards CP reduction, with odds ratios (OR) for the development of CP ranging from 0.40 to 0.94 (any value below 1 exemplifies reduced odds of CP) [106–109]. Pierrat et al. also found an association of reduced CP risk in infants between 22 and 34 weeks of gestation given antenatal magnesium sulfate [110]. The number of pregnant women who need to be treated to have one infant avoid CP have been reported between 29 and 74, with the lower end of that spectrum being those who give birth to more extremely premature infants. It has been reported that antepartum magnesium sulfate for infants born under 30 weeks gestation can prevent 30% of cerebral palsy [90]. Exceptions are cases of chorioamnionitis, pre-eclampsia, and maternal obesity, where $MgSO_4$ seems particularly unhelpful [109, 111, 112].

The exact dosing regimens, side effects, and implications in practice are still subject to debate.

Different studies have employed varied dosing regimens. In obesity, it has been suggested that higher doses or longer exposure times may be beneficial [114, 115]. However, various studies raised concern of magnesium sulfate exposure

and fetal health [68, 69, 116–119]. In particular, a higher dose must be used with caution after negative effects were found in a randomized controlled trial by Mittendorf et al. in infants having high $MgSO_4$ exposure. In a meta-analysis, this study was grouped with other randomized control trials involving antenatal magnesium sulfate exposure, and overall no statistically significant differences in mortality and extreme outcomes were found associated with antenatal magnesium sulfate [120]. Studies using lower $MgSO_4$ dosing that ranged from 4 to 6 g loading dose and 1 to 2 g/h maintenance infusion did not tend to show these adverse effects (Table 37.1). However, there is not onesuniversally accepted dosing quantity and regimen. A meta-analysis by Bain et al. could not conclude an optimal dose due to differences in RCTs analyzed [121]. Hence, the authors, recommend to use the lowest safe dosage of 4 g loading and 1 g/h of maintenance for up to 24 h without repeats [107, 113]. Reeves et al. [122] recommend a loading dose of 6 g and maintenance of 2 g/h, with repeat as needed, as [104] had tested [104, 122]. A review by Jelin et al. also suggests that units adopt one dosing regimen that falls between 4 and 6 g initial dose with a 1–2 g maintenance dose [123]. A meta-analysis by [120], found that antenatal magnesium sulfate significantly reduced the risk for cerebral palsy and substantial gross motor dysfunction (although no significant effects overall on mortality with CP rates), but could not elucidate a dosing regimen, as there was no optimal loading or maintenance subgroup conferring significant reduction in CP [120]. However, they noted that the mode of administration was IV. Similarly, a meta-analysis by Conde-Agudelo et al. looking at mothers less than 34 weeks of gestational age did not find a standard dosing regimen or difference of mortality in the treated group. They found that antenatal magnesium sulfate was associated with a significantly reduced cerebral palsy and substantial gross motor dysfunction risk [124]. The authors recommend a $MgSO_4$ treatment not exceeding 6 g loading, 1–2 g/h maintenance, and 24-h durations, and should be targeted to women with a high risk of delivery before 34 weeks of (e.g., premature rupture of membranes, labor in active phase, and planned delivery within 24 h). Additionally, Ohhashi et al. found that a lower dosage of less than 50 g magnesium sulfate was associated with lower rates of cerebral palsy and brain damage for infants between 28 and 32 weeks of gestation [125]. Costantine et al. [126] also conducted a meta-analysis of RCTs that paralleled previous findings: no single dosing regimen was determined, but found significant reductions in cerebral palsy, moderate-to-severe cerebral palsy, and death or moderate-to-severe cerebral palsy associated with antenatal magnesium sulfate [126]. Due to the varied results of trials and the potential risks, there is substantial controversy surrounding $MgSO_4$ for neuroprotection. The American SOGC guidelines (2011) that reviewed clinical trials and studies recommend the use of intrapartum magnesium sulfate for women at risk of preterm birth (less than or equal to 31+6 weeks), and identify it as the only known fetal neuroprotective agent [127]. This being said, current clinical practice guidelines from Canada (published in 2019) and the United States (published in 2016) support the consideration of $MgSO_4$ in women at risk of imminent preterm birth, following a dosing regimen that follows one of the larger clinical trials [128–130]. The Canadian guideline supports the use of 4 g intravenous loading dose over 30 min, with or without a 1 g/h maintenance dose until birth of antenatal magnesium sulfate for imminent preterm birth to reduce death or CP risk loading over 30 min for planned preterm birth, ideally within 4 h of the birth [130]. However, in both instances, they do not support repeat dosing, citing insufficient evidence to support this regimen. Similarly, De Silva et al. [131] published recommendations of antenatal magnesium sulfate for fetal neuroprotection to females at risk of imminent preterm birth at less than 32–34 weeks of gestation [131]. Sentilhes et al. [132] published guidelines that recommend antenatal magnesium sulfate to females at risk of imminent preterm birth from 23 to 32 weeks' gestation with a 4 g loading dose and 1 g/h maintenance [132].

The two largest trials, Crowther [107] and Rouse [104], differed in their dosing regimens as Rouse [104] used repeated treatments [104, 107]. However, both used relatively low doses, so, to err on the side of caution.

There have been several less severe maternal side effects reported, including flushing (most commonly), nausea, vomiting, diarrhea, sweating, headache, dizziness, hypotension, tachycardia, muscle weakness, slurred speech, blurred vision, and injection site reactions [121, 133–135]. Although these are considered more minor, they have been reported to lead to significantly fewer mothers continuing the intervention compared to controls [104, 107, 112]. No increased risk of major adverse effects such as maternal death, respiratory arrest/maternal respiratory depression, cardiac arrest, prolonged mechanical ventilation, nor postpartum hemorrhage have been found [113, 120]. Lu et al. explain that maternal toxicity is uncommon with current dosing practices, and patellar reflex occurs at 3.5–5 mmol/L, respiratory paralysis at 5–6.5 mmol/L, cardiac conduction alteration at >7.5 mmol/L, and cardiac arrest at >12.5 mmol/L [135]. Scheans also caution care in administration with $MgSO_4$ due to its narrow therapeutic window [136]. The controversy comes into play with fetal adverse outcomes. Conde-Agudelo et al. did not find significant differences in adverse neonatal outcomes (e.g., major neurologic disability, neurologic impairment, other neurodevelopmental outcome, blindness, and deafness), but did find risk of necrotizing enterocolitis [124]. Mittendorf et al. found an association with the use of

Table 37.1 Antenatal magnesium sulfate supplementation outcomes: completed human studies

Study	Population	Dosage and regimens	CP outcome	Other primary outcome(s)	Outcomes related to adverse effects
Nelson and Grether [137] Can magnesium sulfate reduce the risk of cerebral palsy in very low birthweight infants?			Significant association between magnesium sulfate administration and CP reduction		
Mittendorf et al. [69] Association between the use of antenatal magnesium sulfate in preterm labor and adverse health outcomes in infants		Median dose of 31.9 g	Unable to assess $MgSO_4$ relation to CP due to limitations in the cohort size		Adverse outcomes (grade 3 IVH, PVL, death, and CP) were correlated with 31.9 g median magnesium concentration, and less extreme outcomes (grade 1 IVH) with 2.0 g median dose
Crowther et al. [107] Effect of magnesium sulfate given for neuroprotection before preterm birth: a randomized controlled trial	Delivery <30 weeks	4 g loading dose with maintenance infusion of 1 g each hour for up to 24 h	No statistically significant difference in CP outcome	Statistically significant reduction in substantial gross motor dysfunction and/or combined death in the infants treated with $MgSO_4$	Trends of less frequent adverse effects on infants exposed to magnesium sulfate (not statistically different)
Rouse et al. [104] A randomized, controlled trial of magnesium sulfate for the prevention of cerebral palsy	Risk delivery 24–32 weeks	6 g loading dose with maintenance infusion of 2 g each hour	Decreased frequency of moderate—severe cerebral palsy in the treatment group observed	No statistically significant difference in outcome of death or moderate—severe cerebral palsy between the treatment and control groups	No increased risk of death in group with moderate-severe CP
[97, 113] Magnesium sulphate given before very-preterm birth to protect infant brain: the randomized controlled PREMAG trial		4 g loading dose only		Trends of improved outcomes in infants with antenatal $MgSO_4$ (more specifically?)	No statistically significant reductions in adverse neonatal outcomes (e.g., hemorrhaging or white-matter injury) No major maternal adverse effects
Magpie trial The Magpie trial: a randomised trial comparing magnesium sulphate with placebo for pre-eclampsia. outcome for children at 18 months	Women with preeclampsia	4 g loading dose with maintenance infusion of 1 g each hour for up to 24 h	Non significant reduction in CP (was it a trend ??)	No change in risk of death or disability for these after 18 months	
Chollat et al. [106] School-age outcomes following a randomized controlled trial of magnesium sulfate for neuroprotection of preterm infants				Non-significant trend of less frequency of death, motor dysfunction, behavioral disorders and cognitive difficulties	

Table 37.1 (continued)

Study	Population	Dosage and regimens	CP outcome	Other primary outcome(s)	Outcomes related to adverse effects
Doyle et al. [108] School-age outcomes of very preterm infants after antenatal treatment with magnesium sulfate vs placebo/C.A. Crowther, J.E. Hiller, L.W. Doyle, R.R. Haslam, Australasian Collaborative Trial of Magnesium Sulphate (ACTOMg SO4) Collaborative group, Effect of magnesium sulfate given for neuroprotection before preterm birth: a randomized	Risk delivery <30 weeks	4 g loading dose with maintenance infusion of 1 g each hour for up to 24 h	No significant differences in CP	No significant differences in mortality, motor function and other functional outcomes between the test and placebo groups	
Hirtz et al. [138] Antenatal magnesium and cerebral palsy in preterm infants		6 g loading dose with maintenance infusion of 2 g each hour		Reduced cranial ultrasound abnormalities (associated with white matter disease and CP)	No harmful effects in the treatment group
Gano et al. [139] Antenatal exposure to magnesium sulfate is associated with reduced cerebellar hemorrhage in preterm newborns				Significant association with a lower cerebellar hemorrhaging risk from MRI imaging soon in the treatment group. (soon??)	
Antenatal magnesium sulphate for the prevention of cerebral palsy in infants born preterm: a double-blind, randomised, placebo-controlled, multi-centre trial	Risk delivery <32 weeks		Reduction of moderate-to-severe CP (is this significant?)		
Magnesium sulfate, chorioamnionitis, and neurodevelopment after preterm birth	Premature delivery but ≥24 weeks, with clinical chorioamnionitis		No statistical significance for CP		

antenatal $MgSO_4$ and adverse outcomes in infants, including intraventricular hemorrhage, periventricular leukomalacia, death, and cerebral palsy [69]. Bain et al. [140] found that a slower infusion reduced the flushing and warmth experienced, while Meints et al. [141] did not differences in outcomes for infants with different infusion durations [140, 141]. Of the many studies conducted, there has been quite minimal support for the concept that $MGSO_4$ is linked to increased occurrence of these major adverse perinatal and neonatal outcomes. However, the trial by Mittendorf et al. [69] understandably caused alarm and has led to this intervention being approached with far more caution [69].

Altogether, no flagrant or major adverse effects have been associated with magnesium sulfate for infants and mothers when used in controlled circumstances and, in lower concentrations around a 4–6 g loading dose and 1–2 g/h maintenance dose is what current guidelines support [130].

Corticosteroids

Corticosteroids are predominantly given to mitigate the effects of bronchopulmonary dysplasia and intracranial hemorrhage that often occurs with preterm births [142, 143]. They may also be beneficial for CP prevention and mortality reduction [15]. When the results of seven studies were combined as part of a meta-analysis, one course of corticosteroids (betmethasone or dexamethasone) given antenatally in the context of threatened preterm delivery reduced the risk for CP with a relative risk [RR] of 0.678, (95% confidence interval [CI], 0.564–0.815) [144]. Children born between 23 and 25 weeks who had received one or more doses of antenatal corticosteroids (dexamethasone or betamethasone) developed moderate-to-severe CP in 8.6% of cases, while those who received none developed moderate-to-severe CP in 12% of cases, for an adjusted OR of 0.76 (95% CI, 0.59–0.98). Although this finding was not significant, those treated did

have significantly fewer cases of death, intraventricular hemorrhage, or periventricular leukomalacia [145]. A study of women at high risk of preterm delivery reported that death or neurologic impairment at 1.5–2 years old was similar in those who received one or multiple courses of antenatal corticosteroids, or placebo [146]. It appears that one repeat course of corticosteroids may not add benefit beyond an initial dose in terms of CP outcome. Women at risk of preterm delivery <32 weeks gestation given one course of betamethasone followed weekly by either additional courses or saline placebo had children with similar outcomes in terms of major neurosensory disability by 2 years old. In a study of women at risk of preterm delivery <34 weeks who were given two courses of betamethasone over a week apart versus one course followed by placebo, a similar result was found: no significant differences in CP outcome [147, 148]. Guidelines recommend a single course of corticosteroids between 24 and 34 weeks gestation for mothers at risk of delivery within 7 days to prevent preterm birth [93].

Other: Vitamin K, Phenobarbital

Few other substances have been explored in terms of CP outcome in humans. A meta-analysis exploring vitamin K for prevention of perivenous hemorrhage (PVH), a risk factor for CP, found one study that reported on CP outcomes. In this study, participants received antenatal phenobarbital and vitamin K. The RR for CP was 0.77 (0.33, 1.76) [149]. A later study that explored antenatal phenobarbital alone did not find any significant differences in CP outcomes between exposed and control groups [150].

Conclusions and Future Directions

Amassing sufficient numbers of patients for RCTs to explore the utility of antenatal interventions requires a lot of time, collaboration, and resources. Unfortunately, the lack of standardization and consistent follow-up of CP as an outcome makes it extremely difficult to make meaningful conclusions in this field. For example, many studies did not have a neurologist or pediatrician to make the diagnosis of CP or the diagnosis was made after the study was completed, contributing to many non-significant CP outcomes, if measured at all. It is vital for follow-up studies to go beyond the first few days or weeks of life and explore more long-term outcomes such as cerebral palsy [151]. Furthermore, many antenatal interventions for cerebral palsy prevention have copious animal research studies and findings, but this work has not yet been translated to human studies. Part of this is due to resource limitations, and another part of this is due to the societal fear of giving medication to pregnant women because of potential fetal harm. Perhaps the most notorious example of the latter is thalidomide which was launched as a treatment for morning sickness in 1957. Before it was withdrawn just a few years later, thousands of infants were born with severe congenital defects and some did not survive at all. Although precautions are necessary to keep the public safe from having another disaster like this, the pendulum may have swung too far in the other direction. There is a culture of costly litigation in healthcare and excessive regulations that make collaboration among different hospitals, cities, and countries extremely difficult. These factors ultimately result in dissuading research that could potentially benefit the maternal-fetal unit [151]. Future research in this field directed to human studies is needed to implement antenatal treatments and identity mothers at certain stages during pregnancy for whom these therapies are warranted.

Although antenatal infections and premature birth, major risk factors for CP, can be altered with some of the interventions discussed, this has not yet translated into improved CP outcomes. The current literature does show potential benefits in terms of CP outcome from antenatal corticosteroids. However, the only currently recommended standard of care for neuroprotection is the use of low dose magnesium sulfate. Regardless of the intervention, maternal access to education, social programs, and consistent healthcare is essential.

Section II: Therapeutic Hypothermia

Janette Mailo

Intrapartum events may lead to transiently impaired fetal cerebral blood flow resulting in neonatal hypoxic-ischemic encephalopathy (HIE) [152]. HIE is estimated to occur in 2 to 8 per 1,000 live births and it accounts for substantial neonatal mortality and morbidity worldwide [153]. It is diagnosed shortly after birth in an infant born at or beyond 35 weeks of gestation. HIE is clinically characterized by abnormal neurological status, including altered level of consciousness or seizures, depressed tone and reflexes, and often accompanied by impaired respiration and feeding problems [154]. HIE diagnostic criteria for neonates younger than 34 weeks have also been proposed [155, 156]. To objectively assess the severity of neonatal HIE, most centers use a modified Sarnat score [157]. In many survivors, HIE is followed by a significant neurodevelopmental impairment, including the development of cerebral palsy [158, 159]. Report by the American College of Obstetricians and Gynecologists estimated that acute intrapartum events account for approximately 10–20% of cases of cerebral palsy (Report of ACOG 2014). No obstetrical interventions have been shown to improve long-term neurodevelopmental outcome from HIE; therefore, the focus remains primarily on neonatal neuroprotective care and long-term rehabilitation [152].

The complex evolution of hypoxic-ischemic injury in the developing brain offers time-limited opportunities for neuroprotective interventions aiming to restore normal maturational process [160]. Currently, the only proven neuroprotective intervention for neonates with HIE is therapeutic hypothermia (TH) started within 6 h after the delivery and maintained for 72 h at 33 to 34°C [161]. The original 2005 NICHD trial of whole-body hypothermia showed a reduced risk of death or disability from 62 to 44% at 24 months of age in neonates with moderate-to-severe hypoxic-ischemic encephalopathy treated with therapeutic hypothermia compared with controls [162]. Subsequently, considerable effort and research led to the establishment of TH as the standard of care in neonates with HIE [161–168].

Whether neonates with mild HIE could benefit from TH is controversial and a subject of intense research in the clinical as well as preclinical settings. Although combined data from HIE clinical trials did not show clear benefits of cooling in mild HIE, abnormal neurodevelopment at 2 years of age is seen in almost 25% of these infants [169]. Mechanism of brain injury in mild HIE likely differs from moderate-to-severe HIE, with white matter injury being the predominant pattern in mild HIE, compared to basal ganglia–thalamus involvement seen in moderate-to-severe injury. Preclinical study showed evidence of improved WM injury in animal model of mild HIE [170]. Although a single animal study reported potentially harmful effect of cooling of the healthy brain [171], this has not been reproduced in subsequent animal [172] or human studies [173]. Unfortunately, follow-up studies so far failed to show a significant difference in neurodevelopmental outcome, a current gold standard measure of the cooling outcome, between cooled and non-cooled neonates with mild HIE. In a study including 32 cooled infants with mild HIE, improved magnetic resonance spectroscopy (MRS) biomarkers of thalamic injuries post cooling did not correspond to better neurodevelopmental outcome in cooled compared to non-cooled infants at 18–24 months of age [173]. Whether mild injury can be associated with adverse neurodevelopmental outcome presenting later in childhood remains unclear. Even bigger question is how to identify those 5–10% of neonates who progress from mild-to-moderate HIE later than 6 h after the delivery, and therefore miss the opportunity for TH despite standardized early neurological exam [174–176]. Not to miss infants who could benefit from TH is a major concern for neonatologists, and therefore initiation of TH in mild HIE is not an uncommon practice in many centers as reported by surveys in UK [177], Australia and US [178–180] even though a good quality evidence supporting this practice is lacking. Large multicenter high-quality randomized trials are needed to assess the value of TH in mild HIE [176].

Hypothermia for less than 24 h is not neuroprotective as determined by unchanged biomarker profiles [181], and prolonged hypothermia for more than 72 h does not add additional benefits [182].

Despite the benefits of TH, its multifactorial neuroprotective effects remain incompletely understood. The main hypothesis behind the neuroprotective effects of 72 h of therapeutic hypothermia is that the perinatal hypoxic-ischemic brain injury evolves overtime. The initial hypoxic-ischemic event marks the primary phase of the neuronal injury. Despite some neuronal loss during this phase, many neuronal cells recover when cerebral reperfusion is restored in the latent phase, which is associated by at least partial restoration of cellular oxidative metabolism [183, 184]. Moderate-to-severe initial injury is followed approximately 6–15 h later by a secondary energy failure associated with the mitochondrial collapse [13]. This stage of injury evolution causes most of the cerebral injury and delayed cell death unless the process is reversed [185]. Clinically, hallmark of the secondary injury is the onset of delayed seizures. On a neuronal level, secondary injury is characterized by the decrease in glucose metabolism, damaging effects of accumulating excitatory amino acids, failure of mitochondrial oxidative activity, accumulation of reactive oxygen species, inflammation, cytotoxic edema, and ultimately cell death [13, 186]. At the same time, activation of protective physiological neuronal responses aims to reduce an ongoing cell injury by accumulation of inhibitory amino acids such as gama-amino-butyric acid [187], as well as, an inhibitory neuromodulator adenosine, all playing an important role in delaying the onset and reducing the severity of energy failure [13, 188]. Experimental studies suggest that to achieve optimal neuroprotection, TH has to be initiated during the latent phase but before the secondary energy failure [184, 189–191]. The more severe the initial injury, the more rapid process to secondary energy failure [191, 192]. Therefore, the reperfusion and subsequent hyperperfusion are the hallmark of an impaired autoregulation leading to delayed cell death, and poor outcome [158, 159]. Regions of the brain with the highest metabolic demand and therefore the highest vulnerability to hypoxic-ischemic injury are basal ganglia and thalamus, sensorimotor cortex, brainstem, and cerebellum [193].

Neuroprotective effects of hypothermia are achieved through multiple different mechanisms as the injury continues to evolve. Pre-clinical studies suggest that reduction in cerebral metabolism, decrease in toxic free radicals, inhibition of excitatory amino acids release, as well as prevention against brain–blood barrier breakdown, all play a role in hypothermic neuroprotection [13, 184, 194]. Role of inflammation in the hypoxic-ischemic injury is suspected, and therefore the effect of hypothermic neuroprotection on inflammatory cascade is being intensively studied in vitro and in animal models [195].

Strong experimental evidence supports hypothermic suppression of the microglial activation, suppression of pro-inflammatory cytokines, chemokines and complement factors release, to be an important step in reduction in further exacerbation of the secondary injury [194, 196]. Small prospective single-center study of 20 neonates with moderate-to-severe HIE, 12 underwent TH, showed a significant decrease in serum proinflammatory cytokine interleukin-6 (IL-6) from 12–48 h after birth in cooled group [197]. Another study of 36 term newborns with HIE, 16 of whom had adverse short-term outcome at neonatal intensive care unit (NICU) discharge, showed a good correlation of serum cytokines with outcome measure [198]. The authors suggested pro-inflammatory IL-6 and anti-inflammatory IL-10 as promising potential biomarkers of brain injury in neonates with HIE undergoing TH. In reality, the role of inflammation in HIE extends beyond the secondary injury. Intrauterine inflammation, infections, and chorioamnionitis can predispose newborn brain to hypoxic-ischemic injury by altering its normal homeostasis and therefore changing response to oxygen deprivation [13, 195].

Although TH does not offer direct neuroprotection after the onset of mitochondrial failure and irreversible evolution towards cells death, clinical and pre-clinical studies suggest that hypothermia may still reduce the extent of seizure mediated injury [184, 199]. Importantly, hypothermic suppression of secondary injury is safe and does not impact recovery of normal cerebral homeostasis [184].

Therapeutic hypothermia can be administered as either total body cooling or selective head cooling, both demonstrated safety [163, 165, 200], and similar effects on long-term neurodevelopmental outcome, by reducing death or major neurodevelopmental disability, when compared to untreated controls [150]. The findings were supported by a 2012 Cochrane systematic review and meta-analysis of seven randomized-controlled trials (RCT) of TH including 1214 neonates with moderate-to-severe neonatal encephalopathy [201]. The authors reached the following conclusions: (1) hypothermia improves survival and neurodevelopmental outcome at the age of 18 months in newborns with moderate-to-severe HIE; (2) to prevent death or major disability, the number needed to treat was 6 for neonates with moderate HIE, and 7 for neonates with severe HIE and, (3) total body cooling and selective head cooling were both found to be equally effective interventions in treating newborn with HIE compared to untreated controls [201]. A subsequent 2013 Cochrane systematic meta-analysis by Jacobs and colleagues of 11 RCTs involving 1505 term and late preterm neonates with moderate-to-severe neonatal encephalopathy reached similar conclusions [202].

Neither selective head cooling nor total body cooling is likely to eliminate hypoxic-ischemic brain injury completely. Furthermore, the short-term and long-term effectiveness of both methods have not been directly compared in large multicenter trials, although smaller studies suggest that total body cooling might be more promising in reducing the extent of the brain injury. Single-center retrospective study of 83 infants, of which 49 received total body cooling and 34 received selective head cooling, found that although more than half of the infants in both groups had magnetic resonance imaging (MRI) changes related to HIE between 7 and 10 of life, selective head cooling was associated with significantly higher burden and higher severity of brain injuries [200]. The explanation could be in the gradient of the temperature distribution during cooling. A study using animal model found that while selective head cooling delivers better cooling of the cortex than the deep structures, the whole-body cooling provides more equal temperate distribution across all brain strictures [203, 204].

Although limited in numbers, available long-term follow-up studies demonstrate sustained benefits into at least middle childhood [150, 205]. A 2018 Cochrane systematic review showed high-quality evidence for the effectiveness of therapeutic hypothermia (TH) in the reduction in CP in neonates with hypoxic ischemic encephalopathy compared to standard care, based on 7 randomized clinical trials (RCT), comprising 881 term and later preterm neonates with moderate-to-severe encephalopathy and evidence of intrapartum asphyxia [4]. The number of such neonates needed to treat to prevent one case of cerebral palsy was eight [201, 202, 206]. Long-term follow-up studies will be needed to fully compare the effectiveness of both methods of hypothermia on morbidity and neurodevelopment.

Determining at the time of hospital discharge which neonates are at risk of adverse neurodevelopmental outcome remains a challenge. Clinical exam at discharge has low prognostic accuracy for identification of infants at risk of abnormal neurodevelopmental outcome. Brain MRI after TH is commonly completed within 14 days of life for the purpose of discussing neurological prognosis, since at that time the neuroimaging reliably delineates the extend of the brain injury. However, interpretation of conventional MRI remains subjective even if semiquantitative scoring scales are used. There are currently no standardized early quantitative biomarkers for outcome prediction after TH for HIE. The study by Lally et al was the first to prospectively assess the correlation between thalamic magnetic spectroscopy biomarkers measured after TH but within the first 14 days of life, and outcome of moderate-to-severe disability or death at 2 years of age in 190 infants. The authors showed that thalamic proton MRS could predict adverse neurodevelopmental outcome after TH with the highest accuracy [207], allowing to plan for early intervention and rehabilitation for infants at risk.

Although TH seems to be effective at improving outcomes, many infants with moderate-to-severe neonatal

encephalopathy still remain at risk of death or significant lifelong disabilities despite being cooled, highlighting the need for additional adjunctive neuroprotective treatment strategies and optimization of clinical practice [168].

Advances in perinatal neuroprotection are growing as more therapeutic targets are being identified. Preclinical and early clinical studies of erythropoietin (Epo) suggested its potential for neuroprotection and reduction in impact of an early brain injury. Epo is required for normal brain development. Animal models of HIE demonstrated that Epo attenuates cytokine-mediated inflammation by reducing reactive astrocytosis and microglia activation, and subsequently supports the recovery of neuronal cells and limits the extend of the brain injury [208]. Administration of erythropoietin in extremely low birth infants for up to 1 week after brain injury led to reduction in white matter injury, improved neurogenesis, and axonal sprouting [209]. Neuro-regenerative and neuroprotective effects of erythropoietin appear to be both, short-term and long-term. Short-term effects include anti-apoptosis, anti-inflammatory, neurotrophic, and antioxidant effects. Long-term effects include angiogenesis, neurogenesis, and oligodendrogenesis and improved iron utilization [210]. In a phase II double-blinded placebo-controlled trial, multiple high doses of Epo (1000 U/kg) were given with TH to 24 neonates with hypoxic-ischemic encephalopathy intravenously over 7 days. Authors concluded that Epo appeared safe and resulted in less MRI brain injury and better 12-month motor outcome in infants treated with Epo plus HT compared to HT alone [211].

Preclinical studies of the neuroprotective effects of other adjuvant therapies such as melatonin, magnesium, xenon, allopurinol, and stem-cell therapies also seem promising in pre-clinical studies; however, their impact on neurodevelopmental outcome needs to be further examined in large well-designed clinical trials [168].

Section III: Rehabilitation in Cerebral Palsy

Janette Mailo and Jerome Y. Yager

Early detection of cerebral palsy (CP) in high-risk infants followed by cerebral palsy-specific early interventions should be the standard of care [212, 213]. Over the last decade, we have witnessed significant advances in our understanding of the pathophysiology of early brain injuries associated with the development of CP, leading to a shift toward an earlier CP diagnosis.

Cerebral palsy interventions have been expanding rapidly in the last decade, providing newer, safer, and more effective treatment opportunities. Active movements and therapeutical approaches that optimize motor pathways plasticity have been proven essential in preventing loss of cortical connections and preservation of dedicated motor function [214]. Certain complementary medicine approaches have been associated with improved quality of life. New clinical research is emerging as we continue to gain a better understanding of how specific CP treatment affects the outcomes [215, 216].

CP Diagnosis

The diagnosis of CP can accurately be made before 6 months of corrected age [217].

However, earlier diagnoses of CP in very young infants and especially in neonates remain challenging. Clinical exam at discharge from the hospital has a low prognostic accuracy for the identification of infants at risk of adverse neurodevelopmental outcomes [213]. Brain MRI is typically done within 14 days of life in neonates with suspected brain injury to assess neurological prognosis since it can early and reliably delineate the extent of the brain injury [218]. However, there are currently no standardized early quantitative biomarkers for outcome prediction after an early brain injury, and the interpretation of conventional MRI remains subjective even if semiquantitative scoring scales are used [213, 217]. The study by Lally et al was the first to prospectively assess the correlation between thalamic magnetic spectroscopy biomarkers measured within the first 14 days of life and the outcome of moderate-to-severe disability or death at 2 years of age in 190 infants with moderate-to-severe hypoxic-ischemic encephalopathy (HIE) after therapeutic hypothermia (TH). The authors concluded that thalamic N-acetyl aspartate (NAA) could accurately predict adverse neurodevelopmental outcomes in neonates with HIE treated with TH [207].

Therapeutic Interventions

The purpose of the early interventions in children with CP is to reduce the disability and maximize independence in daily functional activities. Interventions should be selected to address a child's or caregiver's specific goals [215]. If multiple goal-limiting factors are present, a combination of interventions might be beneficial resulting in a better overall functional outcome [219].

High-quality evidence supports the effectiveness of early intensive interventions involving physical and occupational therapies for infants at risk of CP [220]. We know that in infants without an early brain injury, the developments of motor pathways lead to the development of normal muscles and bone growth, which in turn leads to normal maturation of motor behaviors, via exploration and interaction with the environment [152]. However, in children with

or at risk of CP, no standardized early interventions currently exist despite the growing evidence for a critical time window for activity-dependent neuroplasticity affecting corticospinal tract development during the first few years of life, which could have a significant impact on improved functional outcomes [221, 222]. Intervention during this unique period of neuronal plasticity could limit any consequences of an early brain injury to an extent not possible by later interventions, specifically, by preserving the normal pattern of development of descending motor pathways [223].

High-quality trials for early motor intervention are lacking; therefore, therapeutic approaches are based on evidence from small trials and published reviews. Small sample sizes, a variety of the age spectrum, heterogeneity of the interventions, and outcome measures, as well as the variable length of follow-up make recommendations for clinical practice weak to moderate at best, and therefore clinical decisions must be based on individual patient characteristics and resources available [224, 225].

Before the initiation of any interventions, it should be identified whether CP is unilateral or bilateral since the treatments, as well as long-term musculoskeletal outcomes, differ [152]. Available evidence highlights the importance of activity-based therapies with objectively measurable goals, including action observation and goal-directed training [219, 226, 227], bimanual training [228, 229], constraint-induced movement therapy [230, 231], mobility with a focus on gait training [232, 233], and rehabilitation programs including resistance training and adjunct therapies following botulinum toxin injection. The effective therapies take advantage of age-dependent motor pathways plasticity using real-life activities in the form of high-intensity active movements generated by a child and working towards the goals set by caregivers or children themselves [234].

Motivation, engagement, and persistence are vital modulators of neuroplasticity and successful therapies include tasks that are rewarding and enjoyable even for a young child to achieve the desired goal. Child-focused, context-focused, and regular therapies delivered in rehabilitation programs by experienced physical and occupational therapists are all equally effective in increasing mobility and motor function, and the optimal approach depends on the child's individual situation rather than the type of therapy alone [235]. Early environmental enrichment is another promising approach promoting brain injury recovery and improvement of cognitive and motor outcomes. Infants with any type of CP who receive the Goals-Activity-Motor Enrichment (GAME) therapeutic approach, which is an early, intense, enriched, task-specific, training-based intervention at home, have better motor and cognitive skills at one year of age than those who receive the usual care [225].

Further research will be needed to elucidate optimal enrichment interventions for individual situations since the studies vary significantly in design and focus.

The intensity and length of the early intervention are presumed to influence the neurodevelopmental outcome [220]. The best approach to stimulate motor and cognitive development in infants with CP or at high risk of CP likely includes a multimodal approach involving stimulating and supportive but challenging and intensive trial and error repetitive learning as well as promoting supportive parent–child interaction towards a desired goal [220]. Specific therapeutic techniques with emerging evidence for outcome improvement include child-initiated movements, specific task-oriented goals, and enriched environmental modification [224].

In children with unilateral CP, the arm and hand are often more affected than the leg, resulting in limitations in daily bimanual activities, and negatively affecting functional independence at home, in school, and during community activities [236]. Early intensive activity-based, goal-directed interventions, including constraint-induced movement therapy (CIMT) and hand-arm bimanual intensive training (HABIT), are more effective than standard care in improving upper limb function and individualized outcomes [237]. Classic CIMT (CMT) involves full arm casting of the unaffected limb for 21 consecutive days, accompanied by intensive training of the impaired limb for 6 h each day [238]. Modified CIMT (mCIMT) protocols have been developed to make the training more child-friendly, including variations in the type of restraint (glove or sling), the duration of time in the restraint (1–24 h a day), the dose of intervention, and even combination therapies when CIMT is followed by bimanual training [239, 240]. To address limitations of CIMT, such as to enable the practice of bimanual skills, and specifically functional activities that are inherently bimanual, intensive training of bimanual coordination HABIT was developed [239, 241]. CIMT and HABIT appear to be equally effective intensive upper limb training approaches. The gains in upper limb function are reflected in the mode of training; unimanual capacity is improved following CIMT and bimanual performance following HABIT training [242]. Despite increasing evidence for therapies for children with hemiplegic CP, some questions remain unanswered, such as what the most effective interventions are to improve upper limb function in infants under 12 months of age, the optimal duration of intervention, critical threshold dose of intervention, therapy delivery (home, school, clinic, community), whether there is dose-age relationship and who will benefit from intensive therapy remains unclear [243].

There is strong evidence that goal-directed home therapy programs can also be very effective by supporting desired learning in natural settings; and in a well-supported home setting, home therapies can supplement therapies administered in rehabilitation facilities to further expand

exposures to intervention [152, 237]. However, that requires the development and support of new rehabilitation ideas and dissemination of intervention protocols to overcome the knowledge translation barrier for therapists administering innovative interventions, especially outside the rehabilitation facilities [224].

More recently, new clinical research seemed to uncover further potential for improving neurodevelopmental outcomes specifically in children with hemiparetic CP by combining traditional manual therapy with non-invasive brain neurostimulation [244]. Emerging clinical research trials of repetitive transcranial magnetic stimulation (rTMS) in children with hemiparesis reported favorable safety and promising efficacy [236, 245, 246]. In a study by Kirton et al. including children with perinatal stroke, the addition of transcranial direct current stimulation (tDCS) to CIMT and bimanual training was associated with greater subjective, although not objective possibly due to relatively small numbers, measures of motor function while appearing to be safe and well-tolerated [247]. The mechanism by which tDCS might facilitate motor learning in children with hemiparetic is incompletely understood but it likely relies on an additional targeted stimulation of the corticospinal motor tracts [236].

A recent systematic review by Novak et al. analyzes and summarizes effective and ineffective interventions aimed to prevent and treat CP in children [219].

Several potentially promising new rehabilitation interventions have been recently proposed but most of them need more research and large trials to further explore their effectiveness and the specific population of children with CP that can benefit the most. Those that have some evidence of their effectiveness include virtual reality gaming, hippotherapy [248–250], and electrical stimulation [251, 252]. Systematic reviews and small clinical trials in complementary and alternative medicine interventions point specifically to the positive effect of acupuncture [253, 254] and animal-assisted therapy [248, 255]. Other trials concluded that manual therapy relying mostly on passive motor interventions should never be the sole therapeutic intervention since it lacks child-initiated movement and child-initiated problem solving which directly and actively involves a child's motor pathways, and therefore these approaches are only minimally or not at all effective [256].

Even though some of the therapeutic approaches might not directly improve motor function in children with CP, they might still have other important benefits that can improve the overall quality of life. For example, while conductive education does not specifically improve motor function, it can positively advance a child's social skills [257], and reflexology reduces constipation even though it does not appear to enhance motor learning [258]. Massage can significantly reduce pain and improve constipation [259, 260] and yoga can improve attention, muscle flexibility, and balance [261].

Tone Management

There are currently no guidelines or evidence-based recommendations for tone management in children with spasticity or dystonia. Spasticity management should ideally start as early as CP is diagnosed to prevent or at least minimize long-term complications. Lack of evidence for spasticity and dystonia management is especially challenging when treating infants under 2 years of age [262]. Heterogeneity of cerebral palsy etiology combined with variable onset and presentation of spasticity in infants complicates the designs and establishment of clinical trials in this population [236].

The goals of tone management include the prevention of muscle contractures, restoration of functional movement, and reduction in pain [262]. Tone management should be multimodal and encompass physical and occupational therapies, orthotics, pharmacological treatment, and surgical options with specific approaches individualized to each child. Although spasticity is the most common tone abnormality in children with CP, dystonia is also frequent, and both can overlap with other tone abnormalities in any of the CP subtypes.

Oral anti-spasticity drugs with the best evidence of effectiveness include baclofen and diazepam. Although the evidence is weaker, dantrolene and tizanidine may also be effective [219, 259]. There is good evidence that injection of botulinum toxin A (BoNT-A) is effective for decreasing muscle over-activity by reducing muscle tone and strength, resulting in short-lived gains in gait and function in some children with cerebral palsy [263]. Injections are generally considered safe, although some risks to the injected muscles in ambulant children and increased risks of systemic adverse events in non-ambulant children must be considered [263]. The optimal timing of the BoNT-A injections remains unclear. Two recent randomized controlled trials showed that the efficacy of every 12-month injection appears to be similar to more frequent injections, supporting the need for a review of the current protocols [263]. Local injections of alcohol and phenol probably also reduce spasticity short term, but the evidence is weak and side effects are common [219, 259].

For children with severe spastic or dystonic CP refractory to medical treatment, intrathecal baclofen (ITB) pump can be considered to reduce muscle tone, improve function and comfort, decrease musculoskeletal deformity, and make caregiving easier [264]. ITB delivers higher drug doses to the central nervous system than can be achieved by oral administration since baclofen has poor lipid solubility. Although only based on low-level evidence, contin-

uous ITB has been shown to be effective in reducing spasticity and dystonia in non-controlled cohort studies [264]. Despite the potential benefits, implantation of an ITB pump is a complex decision; and substantial complications need to be considered since the children eligible for pump implantation are in already vulnerable physical conditions [264]. Many questions remain unanswered, including patient selection, catheter tip placement, optimal dose, and pump flow rate [265].

Selective dorsal rhizotomy (SDR) is a potentially effective neurosurgical procedure aimed to reduce spasticity and improve mobility in carefully selected young children with spastic cerebral palsy by selectively cutting lumbosacral dorsal roots of the spinal cord leading to reduced excitatory input and with that associated decreased muscle tone [266].

Dystonia in children with CP is often under-recognized and high-quality randomized trials of pharmacologic therapies are lacking. Dystonia is characterized by movement-induced, sustained, or intermittent contraction of agonist and antagonist muscles leading to repetitive movements and or twisting posture. Dystonia commonly involves muscles of the limbs, trunk, neck, and face. It can be very painful and lead to difficulties in caregiving and activities. Most of the pharmacological and neurosurgical management is based on clinical expert opinion [267]. Gabapentin may ameliorate the severity of dystonia, ease caregiving, and improve quality of life [268]. Limited evidence shows support for local injection of botulinum toxin in reducing pain and easing caregiving [269]. Oral trihexyphenidyl may reduce dystonic and athetoid involuntary movements, but side effects may outweigh the benefits [270]. For the treatment of severe dystonia invasive therapies including ITB and deep brain stimulation are possibly effective [271]. Botulinum toxin, ITB, and gabapentin appear to reduce pain, further supporting the clinical decision to use these agents as an acceptable intervention for dystonia in CP [219].

Children with CP, more than one tone abnormalities, and multiple comorbidities may benefit from a medication addressing more than one symptom instead of targeting the symptoms individually, such as giving gabapentin to a child with dystonia and epilepsy [267, 268].

Orthotics and Mobility Devices

Orthotics and mobility devices promote function, mobility, and participation in activities for children with CP. These include braces, orthotics, standers, seating systems, and mobility devices.

in a prospective randomized control trial, daytime use of ankle-foot orthoses (AFO) was more effective in improving gross motor function measure scores than day-night use; moreover, prolonged wearing of AFO had a trend to negatively influence muscle activity [272].

Future Directions

Future neuroprotective interventions are expected to take unique advantage of specific stages of fetal and neonatal brain development and advances in biomarkers of brain injury, genetics, and neuroimaging [273]. Neuro-regenerative treatments after early brain injury are a target of intense research.

Transcranial magnetic stimulation has shown to be a feasible intervention to modulate motor learning in children after perinatal arterial ischemic stroke, but specific effects of location and timing need further research [274]. Combined repetitive transcranial magnetic stimulation (rTMS) and constraint-induced movement therapy (CIMT) have been shown to enhance therapy-induced subjective functional motor gains in children with stroke-induced hemiparetic cerebral palsy, a Class II evidence [236].

There might be a potential for individualized rehabilitation based on measurable differences in plastic reorganization. However, the timing of the therapy is important because there might be a period in the early development when interventions might be of maximum benefit [275]. In addition, with a better understanding of how the brain reacts to injury, functional brain imaging may lead to personalized rehabilitation to optimize motor function based on the pattern of plastic adaptation seen in a particular child's brain [236, 244].

Section IV: Transcranial Magnetic Stimulation (TMS)

Janette Mailo

Cerebral palsy is defined as permanent but non-progressive motor deficits [276]. However, focal motor deficits are unlikely to be seen in a neonate. The motor deficits are first noticed around 4–6 months and the extend of the disability cannot be fully appreciated for another several years. Preclinical and clinical evidence suggest that a natural plasticity of the developing brain offers a limited-time opportunity when therapeutical interventions can maximize child's neurodevelopmental outcome [222]. However, despite the growing evidence for a critical time window to maximize activity-dependent neuroplasticity, no standardized early interventions for infants and children with CP currently exist [277].

Two motor cortexes are interconnected through complex neuronal networks between the hemispheres, as well as within each hemisphere. Both types of connections and their complex excitatory and inhibitory circuits are highly involved in plasticity and motor outcome following a focal brain injury [278]. Within the cerebral hemisphere, abnormal motor cortex excitability was associated with a decrease in GABAergic short-interval intracortical inhibition circuits (SICI) in children with hemiparetic CP [279]. Besides the connections within the hemispheres, the interhemispheric connections also play an important role in motor development. For example, via transcallosal networks each motor cortex provides inhibitory effect on the other motor cortex through the interhemispheric inhibition (IHI) of the GABAergic interneurons [280, 281]. The excessive interhemispheric inhibition from unaffected to the injured motor cortex has been associated with worse motor outcome in adult stroke survivors, suggesting a potential target for neuromodulation [282]. Imbalance of the interhemispheric inhibition has also been found in children; however, the effect of developmental changes following an early focal brain injury is not yet fully understood [245, 283]. Modulation of SICI and IHI networks and therefore neuronal circuits within and in-between the hemispheres could potentially enhance neuronal plasticity and improve motor learning in children after early focal brain injuries such as perinatal stroke [278].

During the normal early motor development, contralateral and ipsilateral corticospinal motor neurons initially compete to innervate spinal lower motor neurons. Overtime, the growing dominance of contralateral projections leads to gradual elimination of the ipsilateral inputs and takeover of the motor control by contralateral inputs [284, 285]. Early brain injuries seem to influence this normal contralateral dominance as we can see in children with hemiparesis due to perinatal stroke, suggesting the importance of persisting ipsilateral connections in motor outcome [222].

In children with unilateral CP, the arm and hand tend to be more affected than the leg, resulting in limitation in daily bimanual activities, and therefore negatively affecting functional independence in home, at school, and during recreational activities [237]. Early, intensive activity-based and goal-directed interventions, including constraint-induced movement therapy (CIMT) and hand arm bimanual intensive training (HABIT), have been shown to be more effective than standard care in improving upper limb function and individualized outcomes [243]. However, despite intensive activity-based rehabilitation, significant motor disability persists in many children highlighting the need for other interventions that could further enhance early neuroplasticity.

Carefully timed and focused motor intervention during the unique period of brain plasticity could then influence early corticospinal tract maturation by preserving the normal developmental pattern of descending motor pathways and lead to an improved long-term functional outcome to an extent not possible by any later interventions [221].

Neuromodulation has a potential of such intervention, although its rehabilitation potential in children with hemiparetic CP is still underrecognized [247, 286–288].

In animal studies daily electrical stimulations of motor cortex preserved what normally would be damaged corticospinal projections, after just a few applications [289–291].

As shown in previous histopathological [292], animal [290] and human studies [293, 294], targeted neuromodulation by stimulating contralateral, injured, motor cortex, or inhibiting ipsilateral, non-injured, motor cortex could potentially improve motor function in children with hemiparetic CP by restoring more physiological pattern of corticospinal connections [285].

Neuromodulation is a promising intervention that has been shown to enhance motor learning in adults with stroke [295–299]; however, studies in children remain sparse [172]. Although strokes in adults and children have different pathophysiological mechanisms, basic principles of brain stimulation in children with hemiparetic CP can be extrapolated from the studies in adult stroke survivors, allowing for application of the newer neurostimulation techniques including transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) in children with perinatal stroke [222].

Improved motor learning following transcranial direct current stimulation (tDCS) therapy was first demonstrated in adults (Class I evidence), but more recently studies in children with hemiparesis have been emerging [247]. tDCS consists of 2 scalp electrodes generating weak direct current of 1–2 mA leading to dose-dependent modulation in cortical excitability that can be excitatory or inhibitory depending on polarity [300, 301].

The side effects are mild and include mild transient paresthesia. In a randomized, controlled double-blind clinical trial, 1 mA cathodal tDCS over the non-lesional primary motor cortex was added to moderate-dose motor learning therapy in 24 children with perinatal stroke [247]. The authors proved safety and feasibility of the procedure but no significant improvement in objectively measured motor function [247]. Considering significant subjectively reported gains in motor function by the study participants, the authors hypothesized that the underdosing of the therapy could be one of reasons for the lack of objective motor gains [247].

Neuromodulation therapies could be technically difficult to apply at the time of their highest expected therapeutic benefits, that is during infancy and early childhood. Therefore, perhaps even more suitable than tSCD, specifically for pediatric patients, could be transcranial magnetic stimulation (TMS).

The targets for neuromodulation are similar for tCDS and TMS, and include excitatory stimulation of the injured (lesioned) motor cortex, or inhibition of the non-injured (not-lesioned) motor cortex with the ultimate goal to enhance stronger contralateral motor control [302].

During TMS, a strong magnet generating static magnetic field is held over the patient's head covering the cortex area of interest and evoking focal cortical depolarization [303–305]. In adults, just a short-term application, under 30 minutes, was shown to decrease excitability of the primary motor cortex and improve implicit motor learning tasks [306]. The effect on cortical excitability seems to be higher with stronger magnets and longer treatment periods; treatments for up to 120 min have been safely administered [307]. Safety of magnetic stimulation can be implicitly extrapolated from the decades of using MRI with much stronger magnetic fields. Safety was further highlighted by the International Commission on Non-Ionizing Radiation Protection which concluded that there was no evidence of harm with exposures up to 8T (2009).

A few different TMS approaches have been described. Simple TMS techniques measure single-pulse outcomes, such as motor evoked potentials, between cortex and muscles or motor threshold and stimulus response curves reflecting excitability of motor cortex [295, 308]. Advanced techniques include paired-pulse TMS when multiple stimuli are administered in quick succession, providing information of the effect of the conditioning initial stimulus on the subsequent test stimuli and therefore allowing to assess interaction between brain regions within hemispheres as well as between hemispheres [303, 309].

Combining TMS with neuroimaging modalities such as diffusion tensor imaging (DTI) and functional magnetic resonance imaging (fMRI) provides further understanding of the developmental plasticity in children with hemiplegic CP through the analysis of corticospinal tract integrity and functional network connectivity respectively [222]. Changes in functional networks can potentially become much needed biomarkers of active therapy induced changes and/or neurostimulation-induced plasticity after early brain injury [310], which could lead to personalized rehabilitation in children with CP.

Randomized double-blind study on transcranial static magnetic stimulation (tSMS) suggested its modulatory effect of on motor learning in 24 children with hemiparetic CP (Hollis). Simplicity and tolerability of tSMS highlights its potential for use in very young children, especially when combined with home-based therapies [274].

Repetitive brain stimulation using TMS (rTMS) has a potential to elucidate long lasting effects on brain function [222]. Animal and human studies showed that rTMS at high frequencies (>5–10 Hz) stimulates motor cortex [311, 312], while repetitive stimulation at low frequencies (~1 Hz) inhibits cortical function [313, 314]. rTMS safety and tolerability in pediatric population have been first demonstrated in a study of 10 children with subcortical stroke [245, 246], which was followed by another trial of 19 children with congenital hemiparesis [315]. Further advancement of rTMS in children was provided by Kirton and colleagues in a study including 45 children with stroke-induced hemiparetic cerebral palsy [236]. The researchers studied added benefits of repetitive transcranial magnetic stimulation (rTMS) to constraint-induced movement therapy (CIMT) within an 80-h intensive child-centered motor learning therapy. The study provided promising Class II evidence for significant improvement of therapy-induced gains with added rTMS to CIMT which was still present at 6 months follow-up, regardless of severity of hemiparesis [236]. The strength of the study was meaningful sample size and disease specificity confirmed by validated brain imaging criteria [236]. The authors hypothesized that lasting motor function gains could be a combination of persisting synaptic changes combined with positive psychological effect and boost in self-confidence [236]. Importantly, no decrease in hand function with non-lesional inhibitory TMS have been seen [222]. Size of the hardware could be a potential limitation which will be addressed as the technology evolves [222]. Reassuringly, seizures due to TMS have not been reported in children even the presence of underlying epilepsy [245, 303].

In summary, the studies on neuroplasticity and neuromodulation are promising; however, the ultimate goal is the translation into a better clinical outcome for children with disabilities [222].

A key concept that emerged from the neuromodulation studies is the need for continuing activity-based motor learning. rTMS, tSMS, and tDCS are unlikely going to improve motor function without ongoing motor rehabilitation therapies. Rather neuromodulation seems to enhance the endogenous mechanisms of plasticity which in turn increase the effect of active therapies leading to improved motor learning [222]. Furthermore, better understanding of the combined effect of active rehabilitation and neuromodulation on early corticospinal development in children with CP can help to individualize therapies with respect to underlying sensorimotor reorganization [316].

Although pre-clinical and now growing number of clinical studies suggest that improvement of the motor control by the injured hemisphere leads to better overall motor function, the exact effect of the neuromodulation on the lesioned and non-lesioned motor cortex, and what are the mechanism behind interventional plasticity leading to improved motor learning remain unclear [284, 317]. Therefore, to further improve rehabilitation therapies and individualize neuromodulation approaches we need a better understanding neurophysiological mechanisms underlying functional improvement.

Considering life-long morbidity and limited therapeutic options for children with CP, combined with safety and feasibility of non-invasive neuromodulation, further trials leading to development of neuromodulation principles and protocols are needed. Focus on specific diseases will facilitate translation of neuromodulation to other forms of CP with opportunities for meaningful gains of motor learning.

Section V: Regenerative Approaches to Treating Cerebral Palsy

Stephanie R. Beldick and Michael G. Fehlings

Introduction

Cerebral palsy (CP) is a non-progressive central nervous system (CNS) disorder that is acquired through brain damage during the sensitive antenatal, perinatal, or early postnatal time periods [318]; It affects about 2–3.5 children per every 1000 live births [319]. CP is a complex disorder with respect to both patient presentation and pathophysiological mechanisms. The relationship between clinical presentation and anatomical brain injury is highly important, as it dictates the severity and extent of injury according to the classic "structure-function" rule. The developing brain is highly susceptible to injury, and so there are many pathophysiological mechanisms that can lead to a CP diagnosis. The limited capacity for blood vessel dilation in the fetus and infant predisposes the developing brain to hypoxic-ischemic (HI) insults [318], making slight abnormalities in cerebral blood flow possibly detrimental. Fetal growth restriction occurs when there is a lack of blood flow from the mother to the developing fetus, resulting in a lack of proper nutrient and oxygen delivery. This leads to improper growth of the fetus, predisposing it to the development of brain malformations, and birth asphyxia [320]. Perinatal stroke is also a contributing factor leading to the diagnosis of CP (most often hemiplegic CP) in approximately 5% of term infants [318], which is often due to lowered blood flow in the placenta and brain, predisposing the circulatory system to thromboses [321]. Prematurity and low birthweight are correlated with higher neurological disability [322], with a high number of individuals born prematurely at risk of developing CP [318]. The above listed mechanisms of injury cause individuals to present with varying severities of functional motor deficits that are often accompanied by cognitive dysfunctions and a host of other comorbidities [323].

Where to Target Stem Cell Therapies

Research in both human and animal models has helped to elucidate the anatomical substrates of injury, which is important when deciding where to target therapeutic interventions and where to measure changes in brain connectivity in response to treatment. Severe ischemia can result in widespread damage to and cell loss in the thalamus, basal ganglia, sensorimotor cortex, hippocampus, and brainstem [324]. Primary neuronal loss of vulnerable cell populations, such as the transient subplate neurons, can also lead to secondary neuronal loss in regions that depend on this cell population for their development [325]. White matter damage is also a focal aspect of both preterm and term injury [180]. In the preterm infant, periventricular white matter injury is common. While the incidence of cystic white matter lesions is declining, diffuse white matter injury remains a leading causative factor for subsequent disability [326]. At the cellular level, insults to a vulnerable sub-population of developing oligodendrocytes leads to cell death as well as cellular dysmaturation [327]. This in turn results in hypomyelination at the sites of important white matter tracts, including the descending corticospinal tract, thalamocortical connections, and corpus callosum [326, 328]. Hypomyelination prevents efficient neuronal signaling that is necessary for further synaptic development and can lead to dysmaturation of neurons and their dendritic arbors [325]. These various regions that can be affected in CP provide a plethora of suitable targets for the application of regenerative stem cell therapies. The goal of stem cell therapies for CP is twofold: replace lost and non-functional cells and provide the remaining functional cells with trophic support.

What We Know About Stem Cell Therapies

There are two characteristics that make stem cells unique from other cells in the body and lend them great potential in the realm of regenerative medicine. First, they have the ability to divide indefinitely in culture, thus maintaining a self-renewable source, and second, they can differentiate into more specified functional cell types [329]. The power of stem cells lies in our ability to maintain an unlimited, reusable pool, as well as to generate and replace the types of cells that are damaged in various CNS disorders. After generating a large pool of stem cells, they can be directed to transform into more specialized cell types, and much research has been done on understanding the correct set of factors required to obtain cell types of interest. In addition, stem cells are able to respond to and modulate injured microenvironments through the release of trophic factors [330].

A plethora of research has gone into studying the efficacy of stem cell therapies in preclinical research and some clinical research for CP. Numerous types of cells can be used, including mesenchymal stromal cells (MSCs), hematopoietic stem cells (HSCs), and neural precursor cells (NPCs). In addition to the different kinds of stem cells, the source of these cells can vary as well. They can be differentiated from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), or taken from adult tissue.

Sources of Stem Cells

Classically, multipotent stem cells of various lineages have been sourced from the pluripotent embryonal stem cells (ESCs) of the embryonic inner cell mass. Ethical concerns have been raised about using ESCs though, as an embryo must be destroyed in order to obtain the cells. In addition, if all cells are not properly differentiated before transplantation, there is a high risk of tumor formation in the patient. Adult stem cells are multipotent and exist in various types of mature tissue. Benefits to using these cells are that they circumvent the ethical concerns associated with using ESCs, and they are already differentiated into the desired cell type. Risk of tumor formation is also reduced. However, issues can arise when transplanting donor cells into the patient, as graft rejection is a major concern in allogeneic transplant paradigms. iPSCs have some unique features that overcome the above problems. iPSCs are pluripotent and thus match the broad differentiation potential of ESCs, allowing them to be used as an ethical alternative to ESCs. Because they are "induced" into a pluripotent state, the somatic cells from

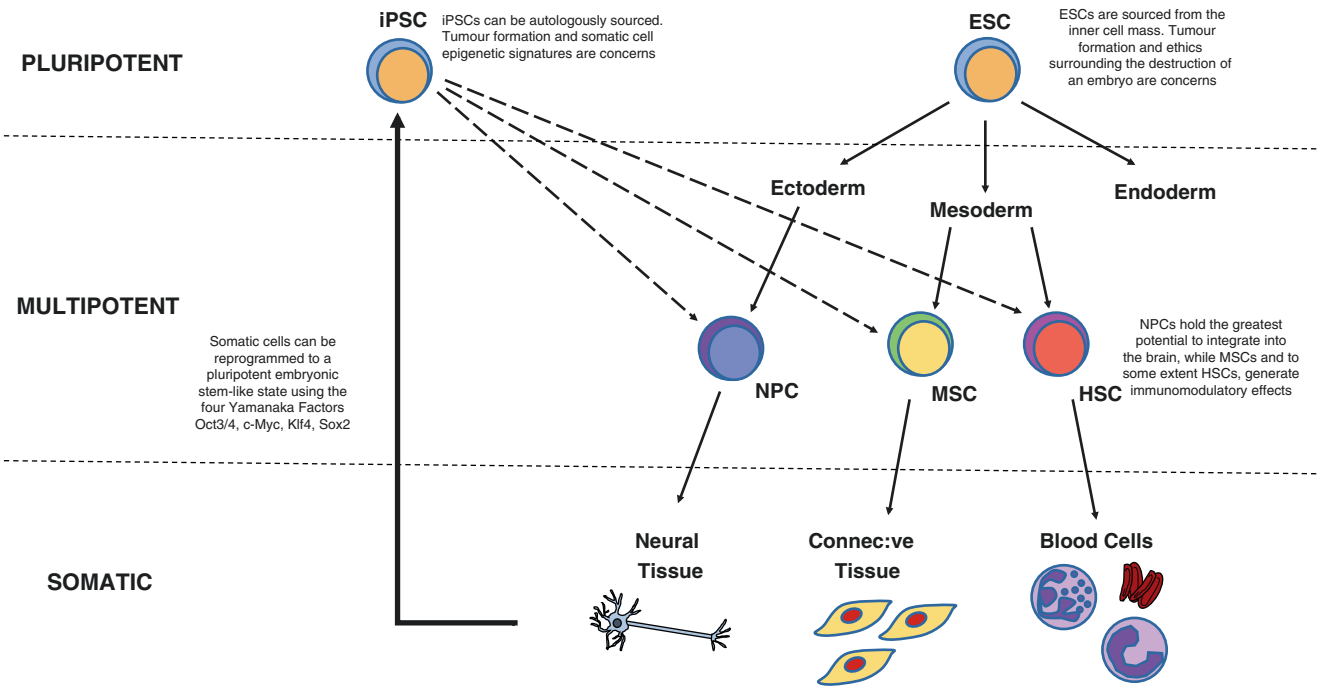

Fig. 37.1 Sourcing of stem cells to treat cerebral palsy

which they originate can actually be sourced from the patient themselves. This drastically reduces the risk of rejection upon transplantation. Still, iPSCs are not without their drawbacks; as with ESCs, tumor formation is a concern. In addition, when somatic cells are induced into the pluripotent state, residual epigenetic signatures from the starting cell population must be taken into consideration when evaluating the utility of the resultant iPSCs and their progeny [329]. Preparing iPSCs from each patient in an autologous fashion can also be quite expensive and time consuming. A summary of the various sources of stem cells is depicted in Fig. 37.1.

Types of Multipotent Stem Cells for Cerebral Palsy

Mesenchymal Stromal Cells and Hematopoietic Stem Cells

When deciding on the type of multipotent stem cell lineage to utilize, several factors should be taken into consideration. MSCs are derived from the mesodermal germ layer, and they have the potential to differentiate into various connective tissue types in the body. Their benefits lie in their easy accessibility and their immune-modulatory properties [331]. However, MSCs lack the full potential to differentiate into functional cells of the CNS. Despite this, MSCs have indeed been investigated in preclinical work. Preclinical studies with MSCs have shown that both single and repeated injections into the injured hemisphere of neonatal HI-injured mice leads to sensorimotor recovery and axonal sprouting [332, 333]. Intranasal administration of MSCs was also found to confer long-term benefits and increased white matter integrity [331, 334]. There are several clinical trials that are currently underway using umbilical cord blood and its stem cell derivatives (HSCs, MSCs, and other progenitors) to treat patients with CP. A randomized double-blind, placebo-controlled trial from Min et al. [335] divided participants aged 10 months to 10 years into three groups: Those receiving conventional rehabilitation therapy only, those receiving recombinant human erythropoietin (Epo) and conventional rehabilitation therapy, or those receiving intravenous allogeneic human-leukocyte antigen (HLA)-matched umbilical cord blood, Epo, and conventional rehabilitation therapy [335]. The study found that the group who received stem cells showed greater improvements on cognitive and motor assessments when compared to the other treatment arms. Sun et al. conducted a randomized double-blind, placebo-controlled crossover trial using intravenous autologous umbilical cord blood in children aged 1–6 years. The researchers found that children receiving higher doses of cord blood had better motor outcomes and increased brain connectivity when visualized on MRI [336].

Similar to MSCs, HSCs found in the umbilical cord blood have immunomodulatory capabilities, though they also possess limited potential to differentiate into cells of neural lineages and integrate into the brain. Preclinical studies in rodents and rabbits using umbilical cord blood have elucidated beneficial effects on behavioral function and structural integrity of the brain [337–339]. The results of the clinical trials presented here suggest that umbilical cord blood has the potential to improve outcomes in children with CP; however, the underlying mechanism behind the improvements have yet to be fully elucidated.

Another clinical trial from Rah et al. [340] took a slightly different approach from using umbilical cord blood, instead using peripheral blood mononuclear cells (PBMCs). The fraction of PBMCs collected from the blood also contains progenitor cells, and the authors note the benefit to using these cells is that repeated collection is possible, unlike in the case of umbilical cord blood. Patients were dosed with intravenous granulocyte colony stimulating factor, which mobilizes the PBMCs and has been shown to have neuro-regenerative potential. The PBMCs were collected and re-administered in an autologous fashion to the patients (aged 2–10 years) 1 month later. The study was conducted as a randomized double-blind, placebo-controlled crossover clinical trial. While some improvement in motor function was noted, along with some changes in fractional anisotropy from diffusion tensor imaging data, the overall results were mild-to-moderate in nature, suggesting that further studies on the utility of these cells are warranted [340].

Neural Precursor Cells

NPCs have unique advantages for regenerating the injured brain in that they are inherently predisposed to differentiate into all cell types found in the CNS. This ability provides NPCs with the greatest potential to integrate into the CNS environment [341, 342]. Despite their potential, NPCs have only been tested to a moderate extent in preclinical models and their only use in a registered clinical trial is still ongoing. Preclinical studies performing transplantations of rodent NPCs into the brains of neonatal HI-injured animals have shown both behavioral and anatomical recovery. Shinoyama et al. [343] induced HI injury in postnatal day (P)2 mouse pups, and two days after injury NPCs were transplanted into the motor cortex. The researchers showed that the transplanted cells differentiated into mature deep-layer cortical neurons and displayed axon outgrowth to various other brain regions, including the corpus callous, striatum, hippocampus, and internal capsule [343]. Park et al. [344] showed that neurotrophin-3 (NT-3)-expressing NPCs transplanted into the infarcted brain region 3 days after injury had reparative effects in a murine P7 HI model [344]. Braccioli et al. [345] induced neonatal HI brain damage in P9 mice and transplanted NPCs into the hippocampus 10 days after injury

Table 37.2 Summary of the pros and cons of various stem cells to treat cerebral palsy

	Mesenchymal stromal cells	Hematopoietic stem cells	Neural precursor cells
Differentiate into neural tissue?	No	No	Yes
Provide trophic support?	Yes	Yes	Yes
Immunomodulatory properties?	Yes	Some	Some
Resident adult tissue?	• Umbilical cord blood • Bone marrow	• Umbilical cord blood • Bone marrow	• Central nervous system
Advantages when derived from ESCs	• ESCs are well-studied		
Disadvantages when derived from ESCs	• Tumor formation • Transplant rejection • Ethical concerns		
Advantages when derived from iPSCs	• Autologous transplants are feasible, therefore low risk of rejection and greater likelihood of higher cell engraftment		
Disadvantages when derived from iPSCs	• Tumor formation • Epigenetic memory		
Advantages when derived from adult tissues	• Low risk of tumor formation • Cells already exist in desired state • Cells are relatively easy to retrieve • Minimal risk of rejection	• Low risk of tumor formation • Cells already exist in desired state • Cells are relatively easy to retrieve • Minimal risk of rejection when umbilical cord blood given	• Low risk of tumor formation • Cells already exist in desired state
Disadvantages when derived from adult tissues	• Limited pool of stem cells • Less limited retrieval if taken from umbilical cord blood bank, but amount of cells varies	• Allogeneic transplant rejection (if given in the absence of umbilical cord blood) • Limited pool of stem cells • Less limited retrieval if taken from umbilical cord blood bank, but amount of cells varies	• Invasive retrieval • Allogeneic transplant rejection • Limited pool of stem cells

induction. NPCs reduced the functional deficits seen in HI animals on the cylinder rearing test. At 56 days post-injury, the brains were analyzed, revealing a rescue of lost brain area, microtubule-associated protein 2 (MAP-2) staining, and synapse number in treated animals. The researchers also showed that NPCs reduced microglia and astroglial activation at 15 days post injury using Iba-1 and glial fibrillary acidic protein (GFAP) staining, respectively. They also showed that NPCs transplanted into the uninjured hemisphere could migrate to the damaged brain regions, suggesting that NPCs are able to hone in on the injured sites via chemotactic factors [345]. A recent study from Rumajogee et al. [346] also present evidence that delayed transplantation of mouse NPCs 2 weeks after P7 HI-injury can lead to motor recovery and restoration of structural integrity in various brain regions. The study focused on the impact of injury and cell transplantation on white matter tracts, specifically in the corpus callosum (the injection site). Electrophysiological data of the corpus callosum suggests that transplanted NPCs helped to restore normal electrical activity, and profiling of co-immunolabeled transplanted cells suggests their mechanism of action may be via providing trophic support, rather than through direct replacement of damaged endogenous cells [346]. Other research has worked to establish preclinical platforms that will facilitate the evaluation of the safety and efficacy of human-NPCs, thereby promoting translation of this therapy to the clinic [347].

In general, NPCs are thought to work through various mechanisms, including replacement of damaged cells and by mediating endogenous cell repair mechanisms through the release of trophic factors such as platelet-derived growth factor alpha (PDGFa) and insulin-like growth factor 1 (IGF-1) [341, 348, 349]. NPCs have been shown to migrate to the site of injury in models of brain injury [350] and promote neuroprotective and anti-apoptotic effects [348]. NPCs have also been shown to modulate the immune system [348, 351].

A summary of the advantages and disadvantages of the various cell types used to treat CP can be found in Table 37.2.

Conclusions

CP is a complex disorder, which causes tremendous impact on those living with the disorder and their community. Conceptually, the major goal of stem cell therapies will be to improve functionality of the brain and reduce motor impairments, thereby improving the quality of life for these patients. However, there is still a lot to reveal about the mechanisms of action of stem cell therapies at both the preclinical and clinical levels. Improving our understanding can in turn help

researchers and clinicians optimize crucial unknowns, such as the best type of stem cell to use and how to modify them for maximal therapeutic benefits, dosing and timing, and which patient sub-population can benefit most from these therapies. Scale-up is another issue which needs to be addressed and is especially important when trying to create uniform therapies out of biologics such as stem cells, which inherently do not exhibit uniformity [352]. Regulatory agencies are still working to develop standardized guidelines for the production and use of each type of stem cell, and this is certainly a limiting factor in bringing stem cell therapies to the clinic for CP [352]. All in all, stem cell therapies hold massive potential for the treatment of CP, and crucial steps must continue to be taken toward understanding how they function and how to optimize their benefits. This is imperative for the future success of stem cell therapies for CP.

Multiple Choice Questions

1. When are the underlying events or injuries that result in cerebral palsy most likely to occur?
   (a) **In utero (during fetal period)**
   (b) During birth
   (c) During infancy
   (d) Early childhood
2. Which of the following therapies is currently accepted as a standard of care throughout the world.
   (a) Transcranial Magnetic Stimulation
   (b) Erythropoeitin
   (c) **Post-Ischemic Hypothermia**
   (d) Stem Cell Therapy
   (e) None of the Above
3. List the types of potential cells available for Stem Cell Therapy
   (a) Mesenchymal Stromal Cells
   (b) Hematopoietic Stem Cells
   (c) Neural Precursor Cells
   (d) **All of the Above**
   (e) None of the Above

   Answers: (a), (c), (d)

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Part V

Co-Morbidities in Neurodevelopmental Disorders

Sleep Disorders

38

Gabrielle Rigney, Pratima Gulati, Penny Corkum, and Shelly K. Weiss

Learning Objectives

At the conclusion of this chapter, the reader will:

- Understand the multiple causes of sleep disturbance in children with NDD.
- Consider the impact that sleep problems have on children with NDD and their families.
- Have updated knowledge on the classification of sleep disorders.
- Develop an approach to the evaluation of sleep problems in this population.
- Learn about a stepwise approach to the treatment of behavioral insomnia.
- Understand the role and lack of evidence for the use of pharmacotherapy to treat insomnia in children with NDD.
- Learn about a transdiagnostic approach to treatment of sleep problems in children with NDD.

Highlights

- There are many different types of sleep disorders, including insomnia disorders, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep-wake disorders, sleep-related movement disorders, and parasomnias.
- Children with NDD have higher rates of sleep problems than their TD peers. Despite some differences in physiological factors involved in sleep being identified for some NDD compared to TD children, the exact relationship between these factors and sleep problems in children with NDD is unknown.
- A range of biopsychosocial factors and their interaction are known to contribute to the etiology of sleep problems.
  - Biological: child's age, circadian factors, and use of medications.
  - Psychological: child's temperament, hypersensitivity to environmental stimuli, comorbid mental health disorders.
  - Social: general parenting practices and expectations, parental mental health.
- There are a range of evaluation tools used for identifying sleep disorders in children with NDD. These tools are the same as those used with TD children, but with some modifications. It is important to consider the type of sleep problem being evaluated, as well as parent or child burden and their ability to comply with the measure.
- A stepwise intervention approach should be used in treating most sleep problems in children with NDD. The four steps include: (1) psychoeducation; (2) healthy sleep practices; (3) behavioral interventions; and (4) medication (if steps 1–3 are not effec-

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tive or the child is in crisis related to their sleep problems).
- Transdiagnostic treatment of sleep problems is recommended, based on commonalities identified in behavioral sleep treatments across NDD diagnoses and in TD children. Specific considerations for children with NDD should be applied to the treatment when required (e.g., more gradual changes for children who are more rigid).

Introduction to Sleep Problems in Children with NDD

A child's sleep behavior is one of the most common concerns among all parents. Evidence highlights that children with neurodevelopmental disorders (NDD) have higher rates of sleep problems and increased vulnerability to the impact of poor sleep than their typically developing (TD) peers. Up to 80% of children with NDD are reported to have disrupted sleep as a comorbidity [1, 2]. For some specific NDD diagnosis, sleep problems are even included as part of the specific NDD diagnostic criteria (e.g., Rett syndrome, Angelman syndrome). Additionally, sleep problems in children with NDD have been found to persist through to both adolescence and adulthood more commonly than in TD children [2]. Sleep problems in children with NDD are largely heterogeneous and multifactorial, with a range of biopsychosocial factors contributing to these sleep problems. These various factors will be explored throughout this chapter.

The brain undergoes extensive neurophysiological changes throughout childhood and adolescence, and thus the consequences of inadequate sleep may be even greater during this stage of life [3]. Of concern is the wide range of deleterious effects that result from poor sleep-in children with NDD. Poor sleep can result in a number of developmental consequences for the child, including difficulties with behavioral and emotional regulation [4], an increase in symptom presentation of the child's NDD [5, 6], and cognitive impairments such as difficulties with attention and memory [7]. The cognitive impairments that result from inadequate sleep can also have an impact on the child's receptivity or ability to benefit from behavioral treatment interventions. It is significant to note that the deleterious effects of poor sleep do not just affect the child. The overall health and well-being of the families of children with NDD who have chronic sleep problems is severely impacted. Given the high prevalence and the known impacts of sleep problems in children with NDD, assessment of sleep problems should be conducted and interventions to address identified sleep problems should be provided.

This chapter will provide the reader with an overview of what sleep is and how sleep differs for children with NDD compared to TD children. This chapter will focus primarily on attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), cerebral palsy (CP), and fetal alcohol spectrum disorder (FASD). These four disorders represent a broad range of NDD in regard to etiology, shared characteristics, and impact on functional capabilities. Sleep disorders will be defined, and the biopsychosocial model will be used as a framework to understand the etiology of common sleep disorders in children with NDD. Following this, current practices for evaluating and treating sleep disorders in children with NDD will be discussed. Finally, the concept of a transdiagnostic approach to treating sleep disorders in children with NDD will be introduced, and details pertaining to the process of developing the transdiagnostic eHealth intervention, *Better Nights, Better Days for Children with Neurodevelopmental Disorders*, will be provided.

What Is Sleep?

Sleep is a reversible state of reduced awareness and responsiveness initiated by inhibition of activating and arousal systems and stimulation of GABAergic neurons [8]. It is essentially a state of resynchronization, an amalgamation of both physiological and behavioral processes [9]. Behavioral factors that influence sleep include the sleep environment (e.g., temperature, light, noise); social and emotional factors (e.g., child temperament, attachment, stress); sociocultural contributors (e.g., socioeconomic status, parenting style); and health components (e.g., medications [10].

Sleep is separated into two broad states of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep. NREM sleep is further divided into three stages from N1 to N3, progressing from light drowsy sleep (N1) to deep slow-wave sleep (N3). REM sleep is the stage most commonly associated with memory of story-like dreams. During REM sleep the brain is highly activated; however, the person experiences muscle atonia, so is unable to move most muscles [11]. Both REM and NREM sleep are thought to help in restoration, with NREM sleep vital in restoration of physiological functions, and REM sleep necessary for restoring mental functions [12, 13].

Electrophysiological recordings, taken through polysomnography (PSG), demonstrate that sleep is a cyclical process, whereby REM and NREM states cycle throughout sleep. This is called the ultradian rhythm. As an example, for a young adult, sleep cycles typically occur between four and six times during a major sleep period, and last for 90–110 min [14]. During the sleep cycles, NREM sleep is most prominent early in the night, and REM sleep progressively lengthens within each sleep cycle. A natural arousal is experienced

at the end of each sleep cycle. Often these are brief and people are not aware of their occurrence [9]. In some instances, these arousals can lead to an extended night waking in children.

Regulation of Sleep

Sleep regulation involves two physiological processes that work together, known as the homeostatic sleep drive and the circadian system. These processes help to ensure optimal alertness during wake, and influence the timing, duration, and the intensity of sleep [15]. The circadian process of sleep regulation promotes sleeping at night and helps to increase alertness during the day, while the homeostatic sleep drive posits that sleep pressure progressively increases during periods of wakefulness and decreases throughout a sleep period [15].

The circadian process is determined by the body's endogenous circadian pacemaker, more commonly known as the "body clock" [16]. The endogenous circadian pacemaker is located in the suprachiasmatic nucleus of the pituitary gland in the brain, and its core function is to regulate daily rhythms to an approximately 24.2 hcycle [16]. The sleep/wake cycle is possibly the most widely recognized of the circadian rhythms; however, other physiological functions such as core body temperature and melatonin secretion are commonly used markers of circadian phase [16, 17]. Circadian processes, including these markers, are also recognized as a key component in the regulation of sleep/wake behavior [16, 18]. The measurement of melatonin, a hormone that promotes sleepiness, is often used as a measurement of circadian phase as its secretion also displays circadian rhythmicity [19].

The regulation of sleep differs in infants and children compared to adults. Infants display irregular daily rhythms due to the circadian timing not being synchronized at birth. However, by 3 months of age, hormonal and sleep cycles consolidate and begin to show a regular 24-h rhythm due to neuronal development and exposure to environmental time cues [20]. The circadian system appears to stabilize from the age of 6 months and is understood to remain that way until puberty [20, 21]. For the homeostatic process, it is believed that for infants and young children the buildup and dissipation of sleep pressure is greater than for adults [22], which may explain the numerous sleep/wake episodes in infants and napping behavior of young children [22]. These developmental changes in sleep regulation are also reflected in the predictable developmental changes that occur in both the quantity and quality of sleep required throughout the lifespan, with sleep need decreasing over the 24-h period with age before stabilizing in young adulthood.

How Sleep Physiology Differs in Children with NDD Compared to TD Children

There is a small but expanding body of research examining how sleep is different in children with NDDs compared to their TD peers. The research conducted to date has often been disorder-specific (e.g., examining sleep in children with a specific NDD compared to TD children). This lack of transdiagnostic research is surprising given that sleep problems are highly prevalent in the vast majority of NDD populations, and that it is likely that similar biological, psychological, and social factors contribute to the development and maintenance of these sleep problems. By definition, children with NDDs have differences in the physiology of their central nervous system (CNS) resulting in altered developmental outcomes. There is evidence of CNS differences that could potentially impact sleep. For example, differences in the GABAergic circuit have been implicated in a number of NDDs [23, 24].

Given that changes in sleep architecture (i.e., the cyclical pattern of sleep shifting between REM and NREM sleep) parallels brain development, researchers have tried to find specific sleep architecture profiles for NDDs [25]. The macrostructure of sleep (i.e., what is assessed via standard PSG recordings; REM and NREM characteristics) has been examined in NDD populations. While some NDDs show a specific sleep macrostructure profile (e.g., William's disorder; [26]), others do not (e.g., ADHD; [27]). This inconsistency in findings across studies is likely due to the heterogeneity within certain NDDs, as well as other issues such as comorbidities and medication treatments that impact both sleep and wake. More recently, researchers have examined the microstructure of sleep (i.e., deeper level of processing of the EEG signals; phasic events such as sleep spindles) and there are some intriguing initial findings. For example, differences in sleep spindles (i.e., bursts of neural oscillatory activity that are thought to play a role in sensory gating) have been found in children with ADHD [28].

There is also emerging evidence for differences in the processes that regulate sleep, particularly the circadian system. For example, there is increasing evidence that there are differences in circadian rhythms in children with ADHD [29], as well as in children with FASD [30]. There is also some evidence that children with NDD may have low levels of melatonin, a hormone regulated by the SCN that is directly involved with the regulation of the sleep–wake cycle. For example, low levels of melatonin secretion have been found in children with ASD ([31]) and Angelman Syndrome [32]. While there are some interesting differences in the physiological factors involved in sleep for some NDDs, the exact relationship between these factors and the sleep problems experienced by children with NDD is unknown.

Case Study 1: Sleep Differences in Children with NDD

Samantha, an 8-year-old girl who was referred due to long-standing sleep problems, was diagnosed with Autism Spectrum Disorder when she was 4-years old. She is considered to be high functioning in terms of her intellectual ability but has significant communication and social interaction difficulties. She was also described as "sticky" as she gets fixated on certain topics, the most recently her fascination is with making "friendship bracelets," which she has made for most of the children at her school. She has experienced significant sleep problems since infancy. Her mother reported that as an infant, Samantha would startle easily, even when sleeping. For example, if someone coughed Samantha would wake up and cry out. She continues to wake easily once asleep. She also has long-standing difficulties with falling asleep and waking too early in the morning. When in bed for the night, Samantha was reported to worry about "everything and anything." She would then start worrying about not being able to sleep and being tired in the morning. She would also report being too hot or too cold and that her PJs were not comfortable. It often takes Samantha over 1 h to fall asleep, and she is not typically asleep before 8:30 pm. Her parents reported that Samantha is often awake before they wake up. When they get up at 7 am, Samantha is making bracelets sitting on her bed. Samantha's parents have tried many ways to help her sleep. They have used a worry jar so that any worries could be quickly jotted down and discussed the next day, have a visual schedule for her bedtime routine, purchased soft PJs and removed the tags, kept her room at a moderate temperature, and have a fan in her room to drown out sounds. They also set up the expectation that she does not leave her bedroom until they are up in the morning. Despite all of these strategies, Samantha still struggles to fall asleep and wakes earlier than they would like her to wake. Her night wakings have lessened since implementing these strategies. They were wondering if they should try Melatonin to help her sleep better.

What Are Sleep Disorders

There are many different types of sleep disorders, with insomnia (i.e., frequent and chronic difficulties with falling asleep, staying asleep, and early morning awakenings that interfere with daily functioning) being the most common in children who are TD as well as children with NDD. The International Classification of Sleep Disorders (ICSD-3) published in 2014 classifies seven major categories which include the following: insomnia disorders, sleep-related breathing disorders, central disorders of hypersomnolence, circadian rhythm sleep–wake disorders, sleep-related movement disorders, parasomnias and other sleep disorders [33]. Examples of disorders in each of these categories are outlined in Table 38.1.

Table 38.1 Examples of disorders of sleep in children with NDD

Sleep disorder grouping	Examples of sleep disorders
Insomnia disorders Insomnia disorders include a frequent and persistent difficulty initiating or maintain sleep	• Limit setting disorder • Sleep association disorder
Sleep-related breathing disorders These disorders are characterized by abnormalities of respiration during sleep. In some of these disorders, respiration is also abnormal during wakefulness	• Obstructive sleep apnea • Central sleep apnea
Central disorders of hypersomnolence The primary complaint in these disorders is daytime sleepiness not caused by disturbed nocturnal sleep.	• Narcolepsy type 1 • Narcolepsy type 2 • Klein–Levin syndrome • Idiopathic hypersomnolence
Circadian rhythm sleep-wake disorders These disorders are caused by alterations of the circadian time-keeping system, its entrainment mechanisms, or a misalignment of the endogenous circadian rhythm and the external environment	• Delayed sleep phase • Advanced sleep phase
Sleep-related movement disorders These disorders are primarily characterized by relatively simple, usually stereotyped, movements that disturb sleep or its onset.	• Rhythmic movement disorder • Restless leg syndrome • Periodic limb movements in sleep
Parasomnias Parasomnias are undesirable physical events or experiences that occur during entry into sleep, within sleep, or during arousal from sleep	• Nightmares • Sleep terrors • Somnambulism (sleep walking) • Confusional arousals • Somniloquy (sleep talking)

Etiology of Sleep Disorders in Children with NDD

Children with NDD may have more than one sleep disorder and more than one etiology for the sleep disorder(s). It is helpful to use a biopsychosocial model to consider the etio-

logical factors for various sleep problems. This model, as described by Tietze et al. [34], provides a framework to understanding the development of a disorder that is a result of complex interactions between biological (e.g., genetic, biochemical), psychological (e.g., behavior, mood, personality), and social (e.g., family, socioeconomic, cultural) factors. In terms of understanding the etiology of sleep disturbances, some common biological factors to consider include the child's age (both chronological and mental age), circadian factors (e.g., differences in biologic cues used to set the circadian cycle such as exposure to light), use of medications that affect sleep/wake cycles (e.g., medications used to treat comorbid mental health disorders such as stimulant medications for attention problems), underlying neurologic or physical problems (e.g., epilepsy, gastrointestinal disease, allergies, eczema, nocturnal enuresis), and differences in neurotransmitters systems (e.g., melatonin). Psychological factors that are often associated with sleep problems/disorders include the child's temperament, hypersensitivity to environmental stimuli, difficulties with self-regulation, communication abilities, and comorbid mental health disorders such as anxiety and depression. There are also many common social factors including parental mental health (e.g., anxiety, depression), general parenting practices (e.g., setting expectations, structure, and routines), parenting practices related to sleep (e.g., bedtime routines, sleeping independent of their child), family composition (e.g., bed or room sharing with a sibling), and parental expectations (e.g., how much sleep is needed for their child).

It is important to also consider the interaction between these factors. For example, all children—including children with NDD—will require less sleep at each successive stage of development. However, a teenager with cognitive delay may not be able to be independent in the evening and therefore will require more parental/caregiver supervision. There may be a mismatch between the time a parent wants a teenager with NDD to settle at bedtime, due to parental fatigue from the demands of providing care for the child, and the actual requirement of sleep for the teenager. It is also important to consider co-morbid sleep disorders. For example, if a child is presenting with insomnia, it is important to consider other sleep disorders both in terms of differential diagnosis (e.g., the insomnia symptoms may actually be secondary to sleep apnea) and comorbidity (e.g., night terrors may be worsened by insomnia). The biopsychosocial framework can be applied to all sleep disturbances in order to understand the contributing factors which will inform the assessment and intervention plan.

Case Study 2: Etiological Factors for Sleep Problems in a Child with NDD

Michael is a 10-year-old boy who lives full-time with his grandmother but stays at his father's house every second weekend. Michael was diagnosed with ADHD and a Learning Disorder when he was 6-years old. His cognitive functioning was assessed as falling within the "Slow Learner" range. He has been treated for his ADHD symptoms with stimulant medication for the past 4 years. A number of medications have been tried, with the most recent one being a long-acting methylphenidate medication. While the medications have been helpful in reducing his ADHD symptoms, he experiences side effects such as decreased appetite during the day and trouble falling asleep at night. Otherwise, Michael is generally healthy with the exception of an allergy to dust mites. In terms of Michael's sleep, his grandmother reported that he has always been a poor sleeper and required an adult present for him to fall asleep up until last year. She explained that Michael resists going to bed and has many "curtain calls." Once in bed he worries about problems that happened during the day, especially if he got into trouble at school. She also noted that his mind races with many thoughts when he is trying to fall asleep. She has found that letting him play on his hand-held video game player helps him relax and not to worry as much. Michael's grandmother's most significant concern about his sleep is that he needs her close by to fall asleep. While she doesn't have to lay down with him anymore, she still needs to be on the same floor as his bedroom. She finds this frustrating as she cannot complete her chores until he falls asleep. She reported that it can often take Michael up to 1 hour from the time he goes to bed to fall asleep, so on most nights he is not asleep until 11:00 p.m. She also noted that it is hard to wake him up at 7:00 a.m. so that he can catch the school bus at 7:30 a.m. She had heard about a herbal remedy for sleep and wanted to know if this might help Michael sleep better.

How to Evaluate Sleep Disorders and Considerations for Children with NDD

The evaluation tools used for determining sleep disorders in children include those that rely on subjective inquiry (e.g., interviews, questionnaires, sleep diaries) as well as those requiring objective measurements (e.g., actigraphy, PSG,

Interviews – interviews are conducted with either the parent or the child (if developmentally appropriate) in order to obtain a subjective, directed and thorough health and sleep history.

Questionnaires – subjective assessment of sleep, which can be either parent-or self-report. There are a wide range of pediatric sleep questionnaires available, however not many have been validated for use in NDD populations.

Sleep Diary – a commonly used subjective measure of sleep, where information about sleep and wake times, night wakings, napping behaviour, feelings of tiredness, and other sleep-related variables are recorded by a parent/caregiver or an adolescent (if able).

Actigrahy – is an objective assessmentof sleep. An actigraph is a motion sensitive device that is most commonly worn on the nondominant wrist, like a watch, for the duration of at least one week. The information collected is downloaded to a computer and displayed graphically to represent sleep/wake as determined by the person's arm movements at night.

Polysomnography (PSG) – is known as the 'gold-standard' of objective sleep measurement, as it is able to detect sleep disorders by monitoring body physiology such as brain waves, eye movements, breathing, muscle tone, and leg movements.

Video EEG – recording a child with a video and electroencephalography (EEG) during sleep is able to detect the presence of interictal (between seizure) abnormal brain waves or seizures.

Fig. 38.1 Sleep assessment measures

overnight VideoEEG) (see Fig. 38.1 for an overview of sleep assessment methods). It is important for the sleep problem being evaluated to be considered when selecting which measure to use, with some sleep disorders (e.g., insomnia) typically assessed with subjective measures, while other sleep disorders (e.g., OSA) may require objective measures. Additional factors that should be considered when evaluating sleep disorders include child and parent burden (time, cost and/or inconvenience), and compliance with the measure (e.g., will the parent be able to record daily sleep diaries, can the child tolerate PSG procedures).

While the assessment tools are similar for children with NDD as for TD children, some modifications may need to be considered. These considerations will be outlined in the description of the evaluation framework to follow.

Screening Assessment

Given the high prevalence of sleep problems in children with NDD and the significant impact on child and family functioning, screening for sleep problems should be considered best clinical practice. While there are no specific screening tools for children with NDD, the BEARS screening tool has been used in this population [35]. This screening tool includes five items that tap into five major domains of sleep, including: **B**edtime problems, **E**xcessive daytime sleepiness, **A**wakenings during the night, **R**egularity of sleep/wake cycles, and **S**noring [35]. Using this screening tool in clinical practice has been demonstrated to increase detection of sleep problems, which in turn would increase assessment and treatment.

Subjective Assessment

Often the first step of the evaluation is to conduct an interview in order to take a thorough history. The history should be taken from the child (if cognition and language skills are appropriate), as well as from the parent/caregiver, as the parent/caregiver is likely to be able to provide information that a child may not be aware of (e.g., snoring behavior; [36]). Information regarding the child's medical history should be obtained, including both physical and mental health symptoms and diagnoses, as this helps to assess for comorbid conditions that may be contributing to sleep disturbance. Prescription and over-the-counter medications should be identified, as these can also have an impact on sleep (e.g., anticonvulsant, stimulant medication, melatonin, and other herbal products). Following this, the clinician should seek information on specific sleep issues, asking questions about sleep quality and quantity, bedtime routines, the sleep environment, and daytime fatigue and functioning [37].

In order to gain further insight into specific sleep issues, subjective measures such as sleep questionnaires, or a sleep diary are often included as the next step in the evaluation. There are a wide range of sleep questionnaires available for pediatric populations. Please see the review by Spruyt and Gozal [38] for more detailed information of 57 pediatric sleep questionnaires reviewed. One of the most commonly used questionnaires for assessing sleep in children with NDD, particularly ADHD and ASD, is the Child Sleep Habits Questionnaire (CSHQ; [39]). The CSHQ is a parent-report sleep screening survey designed for school-aged children ages 4–10 years. This is a retrospective questionnaire asking parents to report on sleep behaviors that occur during a recent "typical" week. There are 45 items covering the major presenting clinical sleep complaints in this age group. However, while it is commonly used, it is important to note that the CSHQ has not yet been validated for many NDD populations [40]. This limitation is being addressed in the field, with the CSHQ recently being modified and validated for children with ASD, reducing it to a 23-item questionnaire with 4 factors [41].

A sleep diary is a commonly used tool when evaluating sleep, where information about sleep and wake times, night wakings, napping behavior, feelings of tiredness, and other sleep-related variables are recorded by a parent/caregiver or child (if he/she has the cognitive skills to complete this accurately). A sleep diary is most commonly completed daily, and typically used for a timeframe of between 1 week and 1 month in order to gather representative data of an individual's sleep patterns and behavior [42]. There are some limitations attributed to the subjective nature of both sleep questionnaires and sleep diaries, such as overestimating total sleep time compared to objective measures [43], and as such these factors need to be considered when evaluating the sleep information collected by these subjective measures.

Objective Assessment

Objective evaluation of sleep includes actigraphy, PSG, and overnight VideoEEG, all of which can present some unique challenges for children with NDD. An actigraph is an actimetry sensor, which looks like a watch, and continually records movement, and in some cases can also measure light exposure. An actigraph is worn on the ankle for infants and on the nondominant wrist for children and adolescents. As such, sensory issues may make collecting information with an actigraphy difficult with ASD populations. However, modifications to the placement of the actigraph device have been successful, where a pocket is sewn into pajamas just below the shoulder seams and the device is placed there, rather than directly onto the skin of the child [44]. Actigraphy allows for prolonged periods of continuous recording within a child's natural environment, and captures sleep parameters such as total sleep time, sleep onset latency, night-waking, and wake-up time [45]. As actigraphy estimates sleep based on movement, it has been found to underestimate night wakings in children with ASD, as they may lie awake silently at night [46], and may also be less accurate for children with ADHD due to an increase in movement throughout the night [47, 48]. Therefore, results should be interpreted taking these limitations into consideration, but actigraphy can be successfully used to assess sleep in many children with NDDs.

PSG is known as the "gold-standard" of objective sleep measurement, as it is able to detect sleep disorders by monitoring physiologic changes during sleep such as brain waves, eye movements, breathing, muscle tone, and leg movements [49]. However, PSG involves an overnight stay at a sleep laboratory, which may be challenging for children with NDD. Similar to actigraphy, the placement of electrodes can pose an issue for children with increased sensory difficulties such as children with ASD [50], or for children with ADHD who may be more active at night and become disconnected from electrodes throughout the night [51]. Due to these problems, it may be difficult to record a typical night of sleep for a child with NDD using PSG. In order to maximize the chance of obtaining a good recording, in some cases, the child is allowed to fall asleep without any monitoring and it is attached after sleep onset. Another strategy would be to have the child bring some familiar things to the sleep laboratory (e.g., a pillow, stuffed animal). In all cases, a parent stays with the child in the sleep laboratory and can help the sleep technologist to have the best chance of a successful recording. The final objective sleep evaluation which is not always included in discussions of the evaluation of sleep is the VideoEEG (electroencephalography). There is a high rate of epilepsy in children with NDD. Both interictal epileptiform discharges as well as clinical and subclinical seizures can disrupt sleep, and therefore this method of evaluation needs to be considered in selected children with NDD.

While objective measures are needed to evaluate for certain sleep disorders, they are not required for many sleep disorders. Relying on objective measures when they are not needed can result in significant delays in assessment and treatment given the limited access to these assessment measures. It can also be costly to the family and/or health care system as these measures, as PSG and VideoEEG are expensive and resource-intensive.

Case Study 3: Assessment of Sleep in a Child with NDD

Peter is a 7-year-old boy with spastic diplegic cerebral palsy who was referred for problems with both sleeping through the night as well as not being able to cooperate with his physiotherapy due to being constantly tired during the day. Peter walks with ankle-foot-orthosis (AFOs) and canes. He does not wear splints at night. He is in grade 2 at school and other than his fatigue at school, his teacher does not have any concerns about his academics. Peter has never been able to fall asleep alone. Since he has been out of his crib, and in a regular bed, either his father or mother lies down with him at night. His parents massage his legs until he falls asleep. Once he falls asleep, after 30–40 min, his parents go into their own bedroom. Every night, Peter wakes up several times, cries out for his parents since he cannot easily get out of bed and go to them, and either his mother or father goes back to his room and lies down with him until he falls back to sleep. Both of Peter's parents feel guilty leaving Peter at night to fall asleep as they know he cannot get out of bed easily on his own. However, they are also worried that his poor sleep habits are resulting in his being tired during the day and not being able to function optimally during the day, both to pay attention at school and to cooperate with his physiotherapist. They were conflicted about using better sleep practices and teaching Peter to fall asleep independently and the need to massage his legs to help him fall asleep. In evaluating Peter's difficulty with sleep, is it important to consider the different methods of assessment—from subjective to objective? Will you use a sleep questionnaire? Will it be helpful to parents to keep a sleep diary for 1–2 weeks and then you can review this with them and see what Peter's sleep is like over time. If parents endorse that Peter snores at night, do you have access to a pediatric sleep laboratory for an overnight study? If not, will you send Peter to an ENT consultant for evaluation?

How to Treat Sleep Disorders in Children with NDD

Treatment of sleep disorders varies depending on the diagnosed sleep disorder(s), any comorbid physical or mental health disorders, as well as other biopsychosocial factors. The best practice treatment for sleep disorders commonly seen in children are reported in Table 38.2, along with some considerations for children with NDD. In this section, we will focus on the treatment of insomnia, as this is the most common sleep problem across NDDs. There are currently no published clinical guidelines for treating insomnia in children with NDD. However, a recent systematic review, which aimed to identify and evaluate the efficacy of parent-delivered behavioral sleep interventions for children with

Table 38.2 Behavioral strategies used to treat sleep problems

Intervention	Description
Unmodified extinction	Infant is placed in bed while awake, left alone until asleep, and night wakings are ignored. Infant learns to self-soothe once realizing that nighttime crying does not result in parental attention
Extinction with parent presence	Parent remains in room during extinction, acting as a reassurance for the child but providing little interaction
Graduate extinction	This involves ignoring negative behaviors (i.e., crying) for a given amount of time before checking on the child. The parent gradually increases the amount of time between crying and parental response. Parents provide reassurance through their presence for short durations and with minimal interaction
Bedtime fading	Operates by delaying bedtime closer to the child's target bedtime. The goal of this treatment is for the child to develop a positive association between being in bed and falling asleep rapidly. Bedtimes can be gradually moved earlier
Stimulus control	Making the bedroom/bed a discriminant stimulus for sleep by only using the bedroom/bed for sleep (not play, time-outs, etc.)
Sleep scheduling	Scheduling regular, appropriate sleep and wake times that allow for an adequate sleep opportunity
Sleep restriction	Restrict time in bed to build sleep pressure and gradually lengthen time in bed as sleep efficiency improves. Contraindicated in youth with parasomnias, seizure disorders, OSA, mania
Cognitive strategies	These strategies are used to address non-productive beliefs about sleep, including the belief that the child cannot change their sleep difficulty. Coping strategies are also included (e.g., relaxation skills such as abdominal breathing)
Relaxation training	Teach diaphragmatic (belly) breathing and progressive muscle relation to reduce arousal. Need to practice regularly before introducing at bedtime
Reward programs	Reinforce healthy sleep practices, appropriate time in bed, etc.

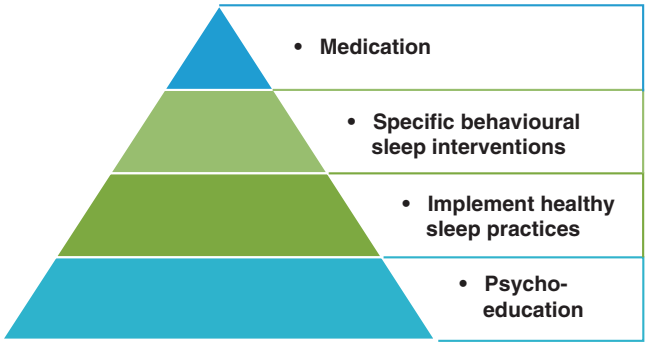

Fig. 38.2 Recommended stepwise approach to treatment of insomnia

NDD, found that there are commonalities in these behavioral sleep treatments both across NDD populations, as well as in TD children [52]. This supports the recommendation that guidelines used in TD children are also suitable for children with NDD. As with the evaluation of sleep problems, the framework for treating sleep disorders remains the same for children with NDD as it does for TD children; however, some modifications may need to be considered. These considerations will be outlined in the description of the treatment framework to follow. Additionally, when treating sleep disorders in children with NDD, an important consideration for clinicians is ensuring that the treatment they provide does not produce or exacerbate other comorbid symptoms.

A stepwise approach is recommended for the treatment of insomnia, which predominantly includes behavioral strategies such as psychoeducation, the implementation of healthy sleep practices, and use of specific behavioral sleep interventions. The last step to consider is pharmacological treatment if the child is not responding to behavioral intervention alone or for short-term use if the sleep problem is considered a crisis for the child (see Fig. 38.2).

Psychoeducation

Although there is very high prevalence of sleep problems reported across the different diagnostic groups of children with NDD, many parents have never sought treatment. This may be due to perceived belief that children may grow out of sleep problems, or that sleep problems are caused by the child's NDD and therefore cannot be treated [53, 54]. It is important that parents are educated about the biology of sleep, consequences of inadequate sleep, and risk factors that may result in sleep disturbance. Psychoeducation is the first step within the model and is a primary component within sleep intervention programs. By increasing one's knowledge and understanding of sleep, an increased level of commitment and motivation to further behavioral sleep strategies may be experienced. There is currently no evidence that indicates sleep education on its own is an effective treatment of sleep disorders in pediatric NDD populations.

Healthy Sleep Practices

Healthy sleep practices (previously known as sleep hygiene) is the second step in the treatment framework and refers to a group of behaviors and environmental changes that aim to reduce factors that are associated with poor sleep and promote factors that are related to improved sleep. Healthy sleep practices have been framed as a mnemonic, the ABCs of SLEEPING [53, 55], which stands for: **A**ge-appropriate; **B**edtimes, wake times and naps, with **C**onsistency; **S**chedules and routines; **L**ocation; no **E**lectronics in the bedroom or before bed; **E**xercise and diet; **P**ositivity and relaxation; **I**ndependence when falling asleep; **N**eeds met during the day...all of the above equals **G**reat sleep! Please see Fig. 38.3 for a detailed description of these healthy sleep practices within the mnemonic. Modifications to each of the letters in the mnemonic may be needed, depending on the child's symptoms and presentation of their NDD. For example, if children are more rigid, then you may consider more gradual changes when developing their bedtime routine [56], whereas children with attention problems will benefit from additional prompts, warnings, and structured routines leading up to bedtime [57].

The ABCs of SLEEPING

	Core concept	Details and Recommendations
A	Age appropriate	It is important that children go to bed and wake up at times that ensure that they receive an age-appropriate amount of sleep. For children who have outgrown naps (which usually occurs during the preschool age period), napping during the day could be an indication that children are not getting sufficient quality and/or quantity of sleep at night.
B	Bedtimes	Having set bedtimes and wake times, as well routines in the evening and morning are key to good sleep. It is recommended that bedtimes be no later than 9pm across childhood.
C	Consistency	It is very important that these bedtimes and wake times are consistent, even on weekends (i.e., no more than 30 min difference between weekday and weekend bedtimes and wake times).
S	Schedule	The child's schedule in general is important – in addition to having routines at bedtime and wake time, it is also important that they have consistency throughout their day, including the timing of homework, extra-curricular activities, etc.
L	Location	It is important that the child's location for sleep includes a comfortable bed, the room is quiet, dark and cool, and the location should be consistent and familiar. Also, the child's bedroom should only be used for sleeping – children should not be sent to their bedroom for a time out. Their bedroom also should not be too exciting or distracting, and should be conducive to relaxation.
E	No Electronics in the bedroom or before bed	The use of electronics, including both the timing of use and the location, should also be considered – children should not be using stimulating electronic devices (i.e., iPods, cell phones, laptops, etc.) too close to bedtime (most commonly defined as one hour prior to going to bed, and it is recommended that these items not be placed in the bedroom.
E	Exercise and diet	Exercise and diet are both important factors that should be considered when evaluating sleep hygiene – physical activity during the day is important to healthy sleep, but should not be undertaken too close to bedtime [defined in the literature as anywhere from 1 hour to 4 hours prior to bedtime. The child's day should be organized so that there is time for a 'cool down' period before bedtime, where they slowly come down from their regular level of activity into a quiet, more restful state. Diet includes things like caffeine consumption – children should limit or totally eliminate caffeine consumption (i.e., pop) – as well as the timing of meals. Children should not be going to bed hungry, but they also should not be consuming a large meal right before bedtime. A healthy balanced diet is also important to the child's sleep as well as to their overall health.
P	Positivity	Positivity surrounding sleep is also an important aspect of sleep hygiene. Parents should have a positive attitude towards sleep and the bedtime/wake time routine, and the atmosphere in the house should be positive, in order to be conducive to creating a positive mood in the child. It is important that this positive mood is relaxing and calming, rather than fun and exciting – we want the child to be winding down before bedtime. Also, doing frustrating activities right before bed (i.e., math problems for a child who struggles with math) is not recommended, as this may interfere with the child's ability to fall asleep.
I	Independence when falling asleep	Independence is also important. Once the child reaches an age where they are capable of settling into sleep without their parents, independence when falling asleep should be encouraged, in order to discourage dependence on someone else in order to fall asleep. For children, independence means no calling out and no getting out of bed, and for parents, no responding to their child calling out and returning the child to their room if they do get out of bed.
N	Needs met during the day	Finally, the needs of the child should be met throughout the day. This refers to both the child's emotional needs (i.e., love, support, hugs, etc.), as well as basic physiological needs (i.e., thirst, hunger, etc.).
G	All of the above equals a Great sleep!	

Bessey, M., Coulombe, A., & Corkum, P. (2013). Sleep Hygiene in Children with ADHD: Findings and Recommendations. *The ADHD Report*. 21(3), 1-7.

Fig. 38.3 The ABCs of sleeping (Reprinted with permission of Guilford Press, Sleep hygiene in children with ADHD: findings and recommendations—the ADHD Report, volume 21, issue 3)

Specific Behavioral Sleep Interventions

The third step in the treatment framework is specific behavioral interventions, which involves applying common psychological strategies within a sleep context. For insomnia, the most common intervention strategies are behavioral strategies such as extinction-based techniques (e.g., unmodified extinction, graduated extinction, and camping out), bedtime fading, stimulus control, and sleep restriction, cognitive strategies, and behavioral reinforcement (see Table 38.2 for definitions of these behavioral strategies). These strategies are based on the principles of behaviorism, which focuses on changing behavioral patterns. Recent reviews of behavioral sleep interventions for children with NDD and chronic health conditions highlighted the need for further research using randomized controlled trial methodology in understudied populations such as CP and FASD [52, 58]. It is encouraging that all studies including children with NDD demonstrated improvements in sleep behavior, suggesting the effectiveness of behavioral interventions in NDD populations [52, 58]. As with the previous steps in the treatment framework, modifications may be required to the interventions dependent on the child's symptom presentation.

Medication

It is known that physicians (including family doctors, pediatricians, and psychiatrists) recommend over-the-counter medications or prescribe a variety of medications to both TD children and children with NDD to treat insomnia, although the rates of medication prescription is much higher for children with NDD [59]. While it is beyond the scope of this chapter to provide a thorough review of the research available in this area, a list of common sedative/hypnotic medications that are prescribed for adult and pediatric insomnia is outlined in Table 38.3 [54, 60–65]. The safety and efficacy data are largely based on studies of adults with insomnia. The side effects listed are commonly seen but not inclusive of all side effects. The use of pharmacotherapy for pediatric insomnia and the vast majority of treatments suggested are "off-label." There is little evidence-based medicine to provide guidance in the use of pharmacotherapy in pediatric insomnia and the review articles and consensus statements are based on expert opinion [66].

In considering the use of pharmacotherapy in children with NDD and insomnia, one can be guided by the consensus statement published in 2005 regarding the use of medications for pediatric insomnia (see Fig. 38.4). Although this consensus statement was developed for TD children with insomnia, it is relevant for children with NDD and remains relevant at the time of the publication of this chapter.

Of particular importance for the NDD population is the use of Melatonin in the treatment of sleep problems, given its high rate of use. Melatonin is an endogenous neurohormone produced predominantly in the pineal gland, is synthesized from tryptophan, and has a circadian rhythmicity. It is thought that the physiological increase in melatonin secretion occurs 1–2 h before bedtime and may be the final trigger for inducing sleep [67]. It is important to understand the two different effects of melatonin, a chronobiotic (i.e., effect on maintaining the circadian rhythm) and a sedative-hypnotic effect. There is scientific evidence that it can be used to shift the biological clock phase in the treatment of circadian rhythm sleep disorders. In this circumstance, the melatonin is given 2–3 h before the dim-light melatonin onset or 3–4 h

Table 38.3 Medications reported to have efficacy for the treatment of insomnia

Class of drug	Examples	Mechanism of action	Common side effects
Alpha 2 agonists	Clonidine Guanfacine	Alpha adrenergic receptor agonist	Clonidine–anticholinergic and REM suppression Guanfacine—Less anticholinergic
Antihistamines	Diphenhydramine Hydroxyzine	H1 subtype receptor agonist	Anticholinergic Tolerance develops with habitual use
Antidepressants	Trazodone Tricyclic antidepressants	5-HT serotonin antagonist	Trazadone-priapism Tricyclic antidepressant-anticholinergic
Benzodiazapine	Clonazepam	GABA receptor	Alter sleep architecture Tolerance develops with habitual use
Nonbenzodiazapine benzodiazepine receptor agonists	Z-drugs	Bind to GABA-A receptor	In adults, reported to cause complex sleep-related behaviors
Chloral hydrate	N/A	Effect due to active metabolite, trichloroethanol but mechanism of action unknown	Not recommended due to adverse reactions, safety and development of tolerance
GABA receptor	Gabapentin	Does not bind directly to GABA, act on calcium channel	Higher doses may cause night waking or difficulty falling asleep

Note: These medications have some efficacy for the treatment of adult insomnia. There is limited research examining their efficacy and side effects in a pediatric population and as such are used "off-label"

Because the ideal pediatric hypnotic does not exist, rational treatment selection should be based on the clinicians' judgment of the best possible match between the clinical circumstances and the individual properties of currently available drugs.

Treatment must be diagnostically driven and based on a careful clinical evaluation of the symptoms and consideration of all possible differential diagnosis.

Medication is rarely if ever indicated in infants and very young children.

In almost all cases, medication is neither the first treatment choice nor the sole treatment strategy.

Medication should always be used in combination with nonpharmacological strategies.

Sleep hygiene should always be optimized before consideration of pharmacological treatment.

Fig. 38.4 Guidelines regarding pharmacotherapy in pediatric insomnia

before desired sleep onset time. In children with NDD and insomnia, exogenous melatonin is used as a hypnotic and potentially to treat insomnia that may be linked to a deficiency in the endogenous production [68]. In this circumstance, it is given close to bedtime.

There have been three systematic reviews of melatonin for children and adults with NDD: one in children with neurodevelopmental disabilities [69], one in children and adults with intellectual disability [70], and one for children and adults with ASD [31]. All three reviews concluded that exogenous melatonin improved sleep by decreasing sleep onset latency, number of night wakings, and increased total sleep time with minimal side effects. There is evidence for the use of melatonin, particularly in children with ASD that shows both safety and efficacy for sleep-onset insomnia. However, there are also unanswered questions regarding melatonin that require further study including dose and formulation. Doses in studies vary widely from 1 to 10 mg. Formulations include immediate release, long acting, or a combination of both. Recently, there has been a prolonged released melatonin with evidence from a randomized controlled trial as well as open label study demonstrating improved sleep onset latency and nighttime sleep duration in children with ASD. This product, Slenyto® has been approved by the European Medicine Agency for pediatric insomnia in children with Smith Magenis Syndrome or ASD [71, 72].

Case Study 4: Treatment of Insomnia in a Child 1 with NDD

Zoe is an 8-year-old girl with FASD who was in foster care for the first 3 years of her life and adopted at the age of 3 years. She was referred for an evaluation of her poor sleep. When her parents first adopted Zoe, they wanted to make sure she was able to become securely attached to them, knowing she had a challenging start in her young life. Night times were always challenging, and it did not seem that Zoe's parents could find the right kind of pajamas and bedding—it was always too hot, or too cold, or too scratchy, or too soft. No matter how hard they tried, it seemed that Zoe's sensory issues became heightened at night. In order to decrease bedtime stress, Zoe's parents got into the habit of turning on the television to Zoe's favorite video and letting her lie between them in the master bedroom, so she would know that her parents loved her and would not abandon her. Once asleep they moved her back to her own bed. Although they knew that it would be better for Zoe to learn to fall asleep without the television and independently, they were worried about her attachment issues and increasing her stress in any way. Zoe's parents were exhausted, as even after she fell asleep, she would wake a few hours later, come into their room, and was only able to fall asleep getting back into the parental bed. Zoe's parents knew that they should teach Zoe to fall asleep on her own and without the television but did not know where to start, and if Zoe would feel abandoned and unloved if they implemented healthier sleep practices. Zoe's parents realize that they have to start with implementing a consistent, short, bedtime routine. The second step will be to use either a bedtime fading or controlled comforting approach to help Zoe learn to settle independently to sleep at bedtime. Considering that Zoe has FASD, which approach would you advise Zoe's parents to try?

Transdiagnostic Approach to Treatment

Historically, there has been a focus on the generation of disorder-specific information rather than an examination of the commonalities across disorders. However, over the past 20 years there is a growing movement toward transdiagnostic conceptualizations with the aim of developing transdiagnostic intervention approaches. The idea is that there are many commonalities across disorders and therefore the same underlying treatment principles can be applied for treatment. It is believed that transdiagnostic interventions will be more resource-efficient, as one intervention could meet the needs of many people with different diagnoses. There is growing evidence in adult populations that transdiagnostic conceptualizations and interventions can be effective for mental health problems [73]. Transdiagnostic research for child mental health is lagging behind that of adult research, but the limited research available is also showing promise for this approach [74].

Sleep has been the focus of attention for psychologists in terms of its suitability to transdiagnostic interventions. Harvey et al. [75] have argued that sleep is mechanistically transdiagnostic, in that some of the etiological factors involved in sleep problems are also involved in the etiology of mental health disorders (e.g., genetics involved in the regulation of circadian rhythms, neurochemical differences involved in the expression of both sleep and mental health disorders). A number of publications have described transdiagnostic interventions for sleep and the scientific underpinnings of these, for adolescents [76] and adults [77]. However, there is limited knowledge about a transdiagnostic conceptualization of sleep in children with or without NDDs. The authors of this chapter have been involved in a pan-Canadian initiative to develop and evaluate a transdiagnostic intervention for treating insomnia in children with NDD (see Fig. 38.5).

Better Nights, Better Days for Children with NDD

Program & Goals:
An interdisciplinary team of Canadian pediatric sleep and NDD experts have developed a transdiagnostic eHealth sleep intervention entitled *Better Nights, Better Days for Children with Neurodevelopmental Disorders* (BNBD-NDD). This program is aimed at children ages 4-10 years with ADHD, ASD, CP, and FASD, and will have the potential to improve the health of children with NDD, as well as the well-being of their families. It is a multi-component, internet-based program that is bilingual (English/French) and evidence-based (http://ndd.betternightsbetterdays.ca/).

Research-Informed Development:
Four preliminary studies were conducted to help inform the modification process of BNBD to BNBD-NDD. These four studies aimed to evaluate the feasibility of a transdiagnostic sleep intervention, by consulting the literature (systematic literature review), external experts in the fields of sleep and NDD (Delphi study), and the families of children with NDD (focus group and usability studies). Overall, key findings from the foundational studies confirmed that transdiagnostic intervention for sleep problems in children with NDD is possible. The findings also demonstrated that eHealth provides a potential solution to some of the current barriers to sleep intervention, and that behavioral interventions used to treat sleep problems in TD children are effective and needed for children with NDD. Important considerations regarding the design of a transdiagnostic intervention for NDD populations highlighted the need for the intervention to be able to address complexity in symptoms and causes, as well as be individualized. The evidence suggests that the required modifications to BNBD were mostly general, however both parents and health care providers may hold ideas that the intervention needs to be NDD-specific. The importance of developing a transdiagnostic intervention, while also ensuring that the needs of each of the specific NDD were met was taken into account throughout the modification process.

Final Program:
A phased approach to the modification process was implemented, combining findings from the four foundational studies, as well as the research and clinical expertise of the BNBD-NDD research team. The first phase is the core program which follows the stepwise treatment model presented above. There are 5 sessions, including Sleep Information, Healthy Sleep Practices, Settling to Sleep, Going Back to Sleep, and Looking Back and Forward. This core program came from the original BNBD program developed for TD children. The second phase provided NDD general modifications such as longer time to complete the program, more support and encouragement embedded in the program, increased recognition of the challenges of parenting a child with NDD, more information about Melatonin and other sleep medications, and modifications to the reward program (e.g., allowing for more immediacy of rewards). Phase 3 involved tailoring the program for symptoms that impact sleep that are pervasive across NDD populations (e.g., anxiety, executive control difficulties, impulsivity) as well as symptoms that are less pervasive but nonetheless still cross-cutting (e.g., pain, medical problems). The last phase was diagnostic specific and was thought to be critical in order to engage parents with this intervention.

Fig. 38.5 Example of a transdiagnostic treatment approach for insomnia in NDD

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Multiple Choice Questions

1. The most common sleep disorder in children with NDD is:
   (a) Obstructive sleep apnea
   (b) **Insomnia**
   (c) Restless leg syndrome
   (d) Sleep terrors
2. Which of the following is *not* considered a psychological factor associated with sleep problems:
   (a) Hypersensitivity to environmental stimuli
   (b) **Parenting practices related to sleep**
   (c) Child's temperament
   (d) Comorbid mental health conditions
3. In order to collect representative data, actigraphy and sleep diary data should be collected for a minimum of:
   (a) 1–5 days
   (b) **1 week**
   (c) 3 months
   (d) 6 months
4. Which of the following is the recommended order for the stepwise approach to treatment of insomnia:
   (a) Medication, behavioral interventions, healthy sleep practices, psychoeducation.
   (b) Psychoeducation, medication, behavioral interventions, healthy sleep practices
   (c) **Psychoeducation, healthy sleep practices, behavioral interventions, medication**
   (d) Healthy sleep practices, psychoeducation, medication, behavioral interventions
5. A transdiagnostic intervention for insomnia in children with NDD is recommended based on all but one of the following reasons:
   (a) **There is clear scientific evidence that transdiagnostic intervention for insomnia is more effective than disorder-specific interventions for insomnia in children with NDD**
   (b) There are many commonalities across NDD in the biopsychosocial factors resulting in insomnia
   (c) Transdiagnostic interventions provide a resource-wise approach to treatment of insomnia in children with NDD
   (d) Transdiagnostic interventions have been demonstrated to be effective in treating insomnia in adolescents and adults, and there is evidence that this approach may also be effective for children

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Optimizing Therapy of Seizures in Children and Adolescents with Developmental Disabilities

39

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Abbreviations

ASD	Autism spectrum disorder
CP	Cerebral palsy
CSWS	Epileptic encephalopathy with continuous spike and wave during sleep
DNMT	DNA methyltransferase
EEG	Electroencephalogram
ESES	Electrical status epilepticus in sleep
FASD	Fetal alcohol spectrum disorders
GABA	Gamma-aminobutyric acid
KD	Ketogenic diet
LKS	Landau–Kleffner syndrome
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NMDA	N-methyl-D-aspartate
PET	Positron emission tomography
SISCOM	Subtraction ictal SPECT co-registered to MRI
SPECT	Single-photon emission computerized tomography
SUDEP	Sudden unexpected death in epilepsy
WHO	World Health Organization

Learning Objectives

- Understand the prevalence and pathophysiology of epilepsy within developmental disabilities commonly associated with epilepsy.
- Identify which children with developmental disabilities are at highest risk for developing epilepsy.
- Recognize the types of epilepsy that occur in children with developmental disabilities.
- Identify the preferred investigations for children with first time seizure and epilepsy.
- Understand the management of epilepsy in children with developmental disabilities.

Highlights

- Children with developmental disabilities are at increased risk of developing seziures and epilepsy regardless of the underlying etiology.
- The phenotype of the epilepsies in children with developmental disabilities is varied.
- Underlying cerebral dysgenesis is the most common reason among the disabilities for resistant focal seizures.
- Children with developmental disabilities, in general, are more likely to be resistant to treatment.
- There are a host of options in the treatment of the resistant epilepsies. Inclusive among these are the ketogenic diet, surgical approaches and neuromodulation.

Introduction: Epilepsy and Developmental Disability

Epilepsy is one of the most common neurological disorders in childhood with an estimated incidence of approximately 44.5/100,000 per year in childhood [1]. The risk of epilepsy in children with neurodevelopmental disabilities is markedly increased compared to the general pediatric population, and the relationship is bidirectional. In a large, population-based study of children with epilepsy, Sillanpaa et al. found that

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39.9% had an additional neurological deficit [2]. In most cases, the underlying etiology for epilepsy is directly related to the cause of the neurodevelopmental disability. There may be structural abnormalities in the brain which may be developmental or acquired. Genetic etiologies are common and increasingly recognized and can also be associated with abnormal brain development. Metabolic disorders may lead to seizures as a result of excessive excitation or deficient inhibition of neurons due to accumulation of certain metabolites, energy deficiency, or neural dysfunction associated with specific structural changes such as storage disorder. Additionally, certain metabolic disorders, including peroxisomal disorders and congenital disorders of glycosylation, can result in malformations of cortical development.

Many children with epilepsy have a complex range of comorbidities including some degree of intellectual disability. The recent ILAE epilepsy classification distinguishes between *developmental* and *epileptic* encephalopathy [3]. The term *developmental* encephalopathy refers to the cognitive and behavioral consequences that arise directly from the effect of the genetic mutation, the metabolic disorder, or the structural abnormality and are neither impacted by seizure frequency nor frequent interictal discharge. Conversely, the term *epilepticencephalopathy* implies that frequent epileptiform activity or seizures further interferes with cognitive development, thus exacerbating the underlying degree of developmental delay. In such cases, improved seizure control and/or significant reduction in the amount of interictal discharge may result in developmental improvement. It is likely, however, that both *developmental* and *epileptic* factors play a role in the encephalopathy of many children, particularly if seizures are frequent and the EEG is very abnormal.

Specific Developmental Disabilities

Cerebral Palsy (CP)

Prevalence and Pathophysiology of Comorbid Epilepsy

Epilepsy is common in children with CP occurring in 20–45% of those affected based on incidence studies ([4–6]). Epilepsy in CP is most frequently due to a structural abnormality with seizures occurring secondary to underlying brain injury or developmental malformations. These structural changes often depend on the underlying cause of CP. In children with CP, the development of epilepsy is most frequently seen within the first year of life [4, 7].

Risk Factors for Development of Epilepsy in Cerebral Palsy

There are multiple risk factors for the development of epilepsy in children with CP. When assessing risk, factors to consider include gestational age, birth history, history of neonatal seizures, and associated comorbid conditions. A Swedish population-based study found that the risk of cerebral palsy and epilepsy was higher when the 10-minute Apgar score was lower, with hazard ratios consistently increasing as the Apgar score decreased [8]. They reported that a 10-minute Apgar score of 3 was associated with a hazard ratio of 425.5 for CP compared to a hazard ratio of 2.4 for a child with a 10-minute Apgar score of 9.

Intellectual disability is also associated with increased risk for seizure in children with CP [4, 6, 9]. The risk of epilepsy is increased in children with spastic hemiplegia or quadriplegia with seizures reported in with up to 50% of children with spastic quadriplegia [5–7]. Conversely, those with spastic diplegia are at reduced risk [7, 10].

The risk for seizures depends upon the underlying etiology. Seizures are more likely to be seen when there are cortical malformations present, a history of intracranial infection, or significant gray matter injury, typically secondary to cerebral infarction [4, 6]. Underlying white matter injury, including periventricular leukomalacia, can also predispose to the development of epilepsy but less commonly than primary gray matter injury [6].

In children with a history of seizures during the neonatal period and cerebral palsy, there is an increased risk for the development of epilepsy [4, 6, 10]. An additional risk is seen in children with dyskinetic CP secondary to thalamic injury [11]. These children are at risk for the development of a unique epilepsy electroclinical syndrome called continuous spike and wave in slow wave sleep (CSWS) which is associated with the EEG finding of nearly continuous activation of the sleep recording (ESES) [11].

Types of Epilepsy in Cerebral Palsy

Given the heterogeneous nature of cerebral palsy, the types of seizures seen can be variable. Reported seizure types include generalized seizure types including tonic-clonic, absence, myoclonic, atonic as well as focal seizures and epileptic spasms [9]. Focal seizures are felt to be reflective of underlying structural brain abnormalities. Children with focal seizures may also have seizures with spread to bilateral convulsive seizure (tonic-clonic) or children can have a combination of generalized and focal seizures [4, 7, 10].

Children with cerebral palsy and a history of neonatal seizures are not only at risk for epilepsy but they are at increased risk for the development of epileptic spasms [4, 10]. Comparing children with CP and epilepsy to a group of children with epilepsy without CP, Hadjipanayis et al. found that the number of children with CP who had epileptic spasms/West syndrome was significantly higher than in their control group [9].

Natural History of Epilepsy Associated with Cerebral Palsy

Multiple studies have reported that the onset of epilepsy in children with CP most often occurs within the first year of life [4, 6, 10]. Children with spastic quadriplegia are more likely to have an earlier onset of seizures compared to those with spastic hemiplegia who may present later in childhood [4]. Some children with cerebral palsy have seizures beginning in the neonatal period and then continue to have seizures throughout life, while others have neonatal seizures with a period of seizure freedom, only for seizures to recur later in childhood or adolescence. In children who develop epileptic spasms, approximately 30% will subsequently go on to develop additional seizure types with progression to Lennox–Gastaut syndrome. Lennox–Gastaut syndrome can commonly be seen in children with CP and a history of CP may portend a more refractory epilepsy course [12].

Factors that may be associated with seizure freedom in children with CP include spastic diplegia, single seizure type, antiseizure medication monotherapy, and normal intelligence [10]. While underlying etiology plays a role, many children with epilepsy and cerebral palsy will have intractable epilepsy with difficult to control seizures and require polytherapy [7, 13].

Management of Epilepsy in Cerebral Palsy

The management of epilepsy in cerebral palsy depends on several factors including underlying etiology and seizure type. In children with epileptic spasms, the recommended therapy includes spasm-specific medications such as ACTH, vigabatrin, and prednisone [14]. In children with Lennox–Gastaut syndrome, or history of tonic and atonic seizures, medications including valproic acid, rufinamide, felbamate, and clobazam can be effective [15]. Adjunctive therapies include the ketogenic diet [16]. In children with refractory epilepsy, surgical options including corpus callosotomy for reduction of tonic and atonic seizures, focal resective surgery, or disconnection surgery are options [17, 18].

Autism Spectrum Disorders

Prevalence and Pathophysiology of Comorbid Epilepsy

The relationship between autism and epilepsy is bidirectional. In a recent review, the rate of epilepsy in individuals with an autism spectrum disorder (ASD) diagnosis was reported to range from 6 to 27% and the risk of autism in children with epilepsy ranged from 5 to 37% [19]. In a study utilizing the National Health Insurance Research Database of Taiwan, Su and colleagues found that the risk of epilepsy was 8.4 times higher in those with ASD than non-ASD controls, and conversely that ASD was 8.4-fold higher in persons with epilepsy compared to nonepileptic controls [20]. Using the U.S. National Survey of Children's Health 2011–12, Thomas et al. noted that epilepsy co-occurred in 8.6% of ASD cases [21].

Accurate diagnosis of epilepsy in a child with ASD can be challenging, as such children may have behavioral staring spells that can mimic seizures, and may have epileptiform discharges on EEG without actual seizures. Clinically, assessment of responsiveness to vigorous tactile stimulation is helpful to distinguish behavioral staring spells from epilepsy.

There are two possible pathophysiological mechanisms which may explain co-existence of ASD and epilepsy [19]. First, both of these disorders may reflect outcomes from common pathological processes, such as excessive excitation or impaired inhibition. Second, epilepsy in early life may impact synaptic plasticity and cortical connectivity, leading to cognitive impairment and autism [19].

Risk Factors for Development of Epilepsy in ASD

The most significant risk factor for epilepsy in children with ASD is intellectual disability. In a meta-analysis of this topic, Amiet and colleagues found that epilepsy rate increased as IQ decreased. Epilepsy was present in 21.5% of subjects with ASD who had comorbid intellectual disability but only 8% of subjects without intellectual disability. Furthermore, those with an IQ < 40 had the highest rate of epilepsy (46%) [22]. Similarly, in 5185 children with ASD older than 10 years of age, Viscidi found that for every one standard deviation increase in IQ, the odds of having epilepsy decreased by 47% [23].

Age is also associated with the development of seizures in persons with ASD. It has been suggested that there are two peaks of epilepsy, one in early childhood and a second peak in adolescence [24]. Several studies have documented that the risk of epilepsy is highest in early adolescence but continues on into adulthood [19, 23].

Specific etiology also contributes to risk of epilepsy. There are several genetic disorders which predispose to both epilepsy and autism, including 1q21 deletions, 7q11.23 deletions or duplications, 15q11.1–13.3 duplications, 16p11.2 deletions, 18q12.1 duplications, 22q11.2 deletions, MECP2 mutations, and TSC1 or 2 mutations [25]. Additionally, metabolic disorders, such as phenylketonuria, also increase risk of comorbid epilepsy.

Other factors that have been reported to contribute to increased risk of epilepsy in children with ASD in some, but not all, studies include female gender, regression of skills, and lower socioeconomic status [19, 21].

Types of Epilepsy in ASD

ASD can be associated with diverse seizure types. Children with West syndrome are at particularly high risk of develop-

ing ASD, with this outcome seen in 9–35% of cases [26–28]. Specific etiologies for spasms (certain genetic or structural causes), severe delay prior to spasm onset, delay in diagnosis, and medical intractability additionally increase the risk. The risk of comorbid ASD is also high with tuberous sclerosis complex, particularly if associated with infantile spasms or temporal tubers [29].

Landau–Kleffner syndrome (LKS) and Epileptic Encephalopathy with Continuous Spike-and-Wave during Sleep (CSWS) may be confused with ASD. However, there are several factors that help distinguish these conditions. First, autistic regression typically occurs early, usually between 18 and 24 months. In contrast, regression with LKS or CSWS usually occurs after age 3 years. Second, LKS affects predominantly language (acquired auditory agnosia), whereas both language and social communication are markedly impacted in ASD. Third, the EEG in LKS or CSWS shows markedly activated epileptiform discharges in sleep, which become nearly continuous. While epileptiform discharges may be seen in ASD, they do not become nearly continuous in sleep. Finally, ASD represents a pervasive developmental disorder. In contrast, cognitive regression in LKS and CSWS may improve dramatically with effective treatment [30].

Natural History of Epilepsy Associated with ASD

Studies have documented that in approximately half of cases, the diagnosis of epilepsy precedes that of ASD ([19]). However, accurate diagnosis of ASD can be very challenging in infancy, and developmental concerns may be attributed to ongoing seizures or side effects from antiepileptic drugs.

There is a broad range in severity of epilepsy associated with autism spectrum disorders. In a study from a single epilepsy center, approximately 1/3 of patients had medically intractable seizures, and earlier seizure onset predicted intractability [31]. There is no clear evidence that autism itself is an independent predictor of outcome in pediatric epilepsy. Rather, outcome appears most dependent on underlying etiology and presence of intellectual disability. Children with underlying cortical dysplasia, significant intellectual disability, and those with specific genetic mutations which impact neural excitability are most likely to remain medically intractable. Underlying epilepsy syndrome is also highly predictive, with much poorer outcomes seen in children with early-onset epileptic encephalopathies, such as Lennox–Gastaut syndrome or West syndrome.

Management of Epilepsy in ASD

Understanding the underlying etiology of ASD and epilepsy is important to identify optimal therapy. Early use of vigabatrin in infants with tuberous sclerosis complex may prevent or attenuate severity of ASD [32, 33]. Similarly, in infants with intractable focal epilepsy, early surgical resection may salvage development [34]. Increased recognition of genetic etiologies may allow for development of precision therapies that target the underlying pathophysiological mechanism.

Medications are typically chosen based on the child's epilepsy type and syndrome. Particular attention should be given to the potential for specific adverse effects. Levetiracetam, perampanel, phenobarbital, and benzodiazepines may exacerbate behavior problems, and topiramate or zonisamide may further worsen verbal skills. Among antiepileptic medications, valproic acid, lamotrigine, levetiracetam, and ethosuximide were perceived to improve seizures the most and worsen other clinical factors the least of all anti-epileptic drugs. Epilepsy surgery should be considered for refractory focal epilepsy and can improve both seizure control, as well as behavior [35]. In children who are not candidates for resective surgery, vagus nerve stimulation or ketogenic diet may reduce seizure burden and potentially may also lessen core symptoms of ASD ([36, 37]).

Cortical Malformations

Prevalence and Pathophysiology of Comorbid Epilepsy

Malformations of cortical development are the most common cause of intractable focal epilepsy in children and many are associated with a genetic etiology [38]. Studies evaluating children with new onset epilepsy have found cortical dysplasias or tuberous sclerosis complex in 1.8–2.4% of cases [39, 40]. In studies limited to infantile onset seizures only, rates of 16–21% are seen [41, 42]. Children with diffuse malformations of cortical development present early in life with global developmental delay, feeding difficulties, abnormalities of head size, other congenital anomalies, and seizures. Those with focal cortical dysplasia limited to a single region may have a normal neurologic examination, a normal intellect or much milder learning disabilities, as well as epilepsy of variable severity. The epilepsy in such cases is typically focal onset [43].

Mechanisms leading to epileptogenicity in malformations of cortical development involve imbalances between excitatory and inhibitory neurotransmission as well as complex interactions between various cell types. There is evidence that NMDA receptors in dysplastic cortex are more hyperexcitable and have decreased sensitivity to magnesium, and there are also higher numbers of non-glutamate receptors. There is decreased density of GABAergic interneurons and possibly, a paradoxical depolarizing effect of GABA. In addition in both focal cortical dysplasia type II and tuberous sclerosis complex there is activation of mTOR and dysmaturity of the cortex [44].

Risk Factors for Development of Epilepsy with Cortical Malformations

The majority of children with malformations of cortical development will develop epilepsy. However, the specific nature and extent of the underlying cortical malformation impacts the age at epilepsy onset, seizure type(s), and risk of intractability.

Types of Epilepsy Associated with Cortical Malformations

The types of epilepsy associated with malformations of cortical development are diverse, but dependent on extent of malformation as well as the age of the child. Diffuse lesions may lead to epilepsies that have both generalized and focal seizures. In such cases, a Lennox–Gastaut phenotype is common. In contrast, localized brain lesions most commonly present as intractable focal epilepsy in older children. Seizure semiology is dependent on topography of the malformation [45]. In infants, however, focal lesions can also lead to an epileptic encephalopathy with generalized seizures and generalized epileptiform discharge [45]. Additionally, infantile spasms, which may be asymmetric, are often the initial seizure type. With increasing age, many children show evolution in semiology from more generalized seizures to more focal ones [46]. Status epilepticus, which can be focal or generalized, complicates the course in between 16 and 30% of cases [46, 47].

Guerrini and Dobyns have classified malformations of cortical development into four groups, based on the main pathway disrupted and the imaging phenotype [43]. *Megalencephaly, hemimegalencephaly variants, and focal cortical dysplasias* are also associated with polymicrogyria as well as changes typically seen with the various types of focal cortical dysplasia. *Tubulinopathies and lissencephalic/double cortex disorders* range from severe lissencephaly and diffuse pachygyria, to less severe malformations in which there is disrupted cortical lamination, radial columnar heterotopia, and ectopic neurons in white matter. Specific mutations of tubulin genes may also lead to ocular and other ophthalmologic abnormalities. *Polymicrogyria disorders* can vary greatly and may be diffuse or more focal, most commonly involving the perisylvian cortex. These disorders are often associated with other malformations of the brain or other organs. Children with bilateral perisylvian polymicrogyria typically present with oral motor dysfunction, intellectual disability, and seizures. Unilateral perisylvian syndrome often presents with mild hemiparesis and focal seizures. *Heterotopia syndromes* refer to the presence of normal neurons in abnormal locations. The most common form of heterotopia is periventricular nodular heterotopia with gray matter nodules lining the ventricular walls and protruding into the ventricles. These changes can also be associated with polymicrogyria, abnormal rotation of hippocampus, hypoplasia of the cerebellum, and other cortical malformations. In patients without associated brain malformations, neurodevelopment can be only mildly affected. However, seizures are common.

Natural History of Epilepsy Associated with Cortical Malformations

The majority of children with focal cortical dysplasia have onset of their epilepsy early in life, with one study documenting that 61% had onset prior to 5 years and 92.5% before 16 years of age [46]. Additionally, in those with early onset epilepsy, a significant proportion began having seizures within the first month of life [45]. Specific underlying pathology is predictive of younger age at onset ([45, 46]). Children with focal cortical dysplasia types IIA and B, which are associated with cytoarchitectural abnormalities with giant neurons and balloon cells, have earlier onset of seizures than those with focal cortical dysplasia type I, which has architectural abnormalities alone [48].

Neurocognitive deficits are very common, particularly with early-onset seizures. In a study of infants with malformations of cortical development and epilepsy onset prior to 1 year of age, 60% were delayed even before seizure onset, and over time further neurocognitive deterioration was seen [45]. Other risk factors for neurocognitive delay include epileptic encephalopathy syndromes, larger lesion size, temporal or posterior location, and longer duration of epilepsy [44, 49].

Malformations of cortical development are highly associated with medically intractability. Most often, seizures are pharmacoresistant from onset although less commonly, transient responsiveness to antiepileptic medication is seen ([46, 47]). Infantile spasms in young infants may respond well to vigabatrin therapy initially, but with time, the majority developed other seizure types which are medically intractable [45].

Management of Epilepsy Associated with Cortical Malformations

First line therapy is typically with anti-seizure medications. While there is no clear evidence of superiority of any particular medication in most types of cortical malformations, vigabatrin, a partial inhibitor of mTOR, and everolimus, a more robust mTOR inhibitor, appear particularly useful in tuberous sclerosis complex. The recently published EXIST-3 trial in patients with tuberous sclerosis aged 2 years and older documented significantly greater responder rates with high-dose everolimus compared to placebo (40% vs. 15%) [50]. There is potential for increased efficacy with time and there is evidence that younger age predicts better response.

While some authors believe that valproate and benzodiazepines may be the most efficacious agents in the treatment of other cortical malformations, others have noted high rates of benzodiazepine insensitivity, or have raised concern that such agents may even be detrimental [49, 51].

Children who fail to gain seizure control with trials of two antiepileptic drugs should be considered for resective epilepsy surgery. Postoperative outcome is often favorable with a meta-analysis of 16 studies, including 469 patients, showing that nearly 60% were seizure-free 1 year after surgery [52]. Outcome is most favorable if a complete lesion resection is possible and if the preoperative MRI shows a clear lesion [53].

In patients with more diffuse or multifocal lesions, palliative surgery can be considered. Corpus callosotomy may be beneficial for patients with intractable drop seizures and Lennox–Gastaut syndrome. Vagus nerve stimulation may also be considered and approximately half of patients will achieve a greater than 50% reduction in seizures with this therapy [54].

A single study examining use of the ketogenic diet in focal cortical dysplasia showed that 61.7% achieved a greater than 50% reduction in seizures by 3 months with nearly 45% being seizure-free [55]. Similarly, studies of the ketogenic diet in tuberous sclerosis complex show that between 47 and 92% achieve a greater than 50% seizure reduction [56, 57].

Fetal Alcohol Syndrome

Prevalence and Pathophysiology of Comorbid Epilepsy

The number of children affected with epilepsy with FASD is estimated to range from 3 to 21% [58]. Boronat et al. performed a prospective analysis of 71 pediatric patients with learning disorders felt to be related to FASD. They found EEG abnormalities in 24.6% of patients and three patients had a diagnosis of epilepsy [58].

The pathophysiology of epilepsy in FASD is likely multifactorial, including direct toxicity of alcohol, altered cellular signaling and structural changes secondary to alcohol exposure. Prenatal alcohol exposure can result in malformations of cortical development secondary to disrupted and abnormal neuronal migration [59, 60]. Additionally, rat fetuses with prenatal ethanol exposure were found to have reduced cortical thickness, felt to be secondary to impaired radial glia, delayed neuronal migration, and reduced number of neuroblasts [59].

Animal models of prenatal alcohol exposure have demonstrated that ethanol given to the developing fetus can result in increased susceptibility to induced seizures [61]. With potentially targeted hippocampal toxicity, ethanol exposure can alter hippocampal physiology and signaling thus promoting epileptic activity and facilitating the development of epileptogenic networks from hippocampal toxicity [62].

Risk Factors for the Development of Epilepsy

The timing of in-uteroethanol exposure may have an impact on the subsequent risk for the development of epilepsy. In a retrospective review of 1063 patients from two FASD clinics, Bell et al. [63] found that 25 patients (5.9%) had a diagnosis of epilepsy and 50 patients (11.9%) had at least one documented seizure. From their review of maternal drinking history, they found a higher incidence of first trimester exposure or exposure during all three trimesters in those patients with seizure [63].

In patients with FASD, given the direct and indirect toxic effects of ethanol on neuronal development and migration, there is increased likelihood of structural brain abnormalities, which can be associated with epilepsy [64, 65].

Types of Epilepsy in Fetal Alcohol Syndrome

Given the variation in neurodevelopmental disabilities and severity in FASD, the epilepsies can be just as varied. In their review of 425 patients with fetal alcohol syndrome and epilepsy, Bell et al. [63] described that the most common seizure types seen in their retrospective review included generalized, absence, and complex partial seizures [63].

Natural History of Epilepsy Associated with Fetal Alcohol Syndrome

Epilepsy in FASD tends to begin in early childhood and adolescence. In a prospective electroencephalographic evaluation of 61 children with a diagnosis of FASD, three children had a diagnosis of epilepsy. Seizure onset in these children was reported to be between 10 months and three years of age and all three children were seizure-free in childhood or adolescence. They found EEG abnormalities in 14 children (23%) with common findings including generalized slow wave abnormalities and generalized and focal interictal epileptiform discharges [58].

Management of Epilepsy in Fetal Alcohol Syndrome

In the children with generalized absence epilepsy, medications including ethosuximide, lamotrigine, or valproic acid are considered first line [66]. Additional options include zonisamide, levetiracetam, and ketogenic diet. In children with atonic and tonic seizures effective therapies include valproic acid, rufinamide, felbamate, ketogenic diet [15, 67]. In children with frequent atonic seizures and Lennox–Gastaut syndrome, then corpus callosotomy can be effective in reducing seizure frequency [17]. In children with refractory focal epilepsy and malformation of cortical development, then epilepsy surgery maybe an option [52].

Investigation of Seizures in Children with Neurodevelopmental Disabilities

Evaluation of all children with potential seizures should start with taking a careful history of the event(s) in question, review of past medical history, and performing detailed general and neurologic examinations. Neurodevelopmental dis-

ability places a child at higher risk for seizures and the seizures can be subtle and difficult to identify in this population [68]. In addition, non-epileptic spells, such as staring episodes or stereotypic behaviors, are frequent and must be distinguished from seizures [69].

The description of the event should include signs or symptoms prior to, during, and after the episode [70]. A history of neonatal seizures is of particular importance, especially for children with CP. In a series of 197 children age 0–12 years with CP, epilepsy was significantly more common in those who had a history of neonatal seizures than those without this risk factor [6].

The examination is important, both to determine whether urgent treatment is required, and also the risk of seizure recurrence and subsequent development of epilepsy. Children with quadriplegia are more likely to develop seizures than those with hemiplegia [6]. Moderate to severe intellectual disability also increases risk [71].

While there may be some recall bias in taking the history, there was 80–99% agreement in responses about specific symptoms during the seizure and 87–100% agreement exam findings when patients were interviewed and examined by two separate physicians [70]. Therefore, the history and exam provide important and reliable information.

In the acute setting, laboratory testing and infectious disease evaluation can be considered. However, this is based on the clinical circumstances of each individual. Toxicology evaluation could also be considered, if there is concern for exposure [72]. Lumbar puncture is of limited value, unless there is a high degree of suspicion for meningitis or encephalitis [73]. The exception to this would be children with ventriculoperitoneal (VP) shunts for hydrocephalus. In these children, seizure with or without fever can be a sign of VP shunt infection and there should be a lower threshold to obtain CSF for analysis [74].

Neuroimaging with CT and/or MRI is also helpful in the evaluation. Neuroimaging should be done emergently if it is felt that the results could affect the acute management, such as identification of tumor, stroke/hemorrhage, trauma, obstructive hydrocephalus, and some metabolic disorders [75, 76]. Therefore, emergent imaging should be performed if there is a new postictal focal deficit that does not quickly resolve or if the child has not returned to baseline within hours [72].

Non-urgent imaging is helpful to identify underlying etiology. Furthermore, if a focal lesion causative of the seizures is found, this may be amenable to surgery in the future [75]. MRI is the preferred imaging modality for children with epilepsy [72]. Indications for imaging include: focal epilepsy, abnormal neurologic exam, developmental delay/arrest/regression, age younger than 2 years, and epileptic encephalopathy. Furthermore, uncontrolled, worsening, or changing seizures or decline in development warrants imaging, even in those with established epilepsy. Finally, seizures or epilepsy presenting with a medical emergency such as status epilepticus also warrant neuroimaging [75].

Electroencephalogram (EEG) is also an essential part of the epilepsy evaluation. A routine EEG is recommended as standard of practice for the evaluation of potential unprovoked seizures [72]. The EEG should include awake, sleep, hyperventilation, and photic stimulation [77]. In children with seizures of unknown cause, abnormal EEG was associated with recurrence in 54%, whereas 25% of children with normal EEG had recurrence [78]. Furthermore, EEG can be helpful in identifying seizure type and epilepsy syndrome. While an EEG done within 24 hours of a seizure is more likely to show abnormalities, focal slowing may be transient, and the presence of an underlying lesion should not be assumed [79]. Furthermore, the absence of abnormalities does not exclude the event being a seizure. Finally, there is no evidence to support performing the EEG prior to discharge from the emergency department [72].

Unfortunately, the routine EEG does not always provide sufficient information to determine whether the events in question represent seizures. This is of particular importance for children with developmental disabilities, such as autism spectrum disorder. Although there is significant overlap between ASD and epilepsy, not all children with ASD and epileptiform EEG have seizures. EEG in children with ASD but without epilepsy demonstrated epileptiform discharges in 50–61% [80]. By comparison, potentially epileptiform discharges have been reported in only 1–4% of healthy individuals [81]. In addition, there does not appear to be a difference in the rate of epileptiform activity based on the presence of regression or behavioral problems [82].

Due to the high incidence of epilepsy in children with ASD, it is important to have an elevated index of suspicion when evaluating spells. Families should be educated about the increased risk of seizures, as well as signs and symptoms thereof [80]. Furthermore, due to the high incidence of abnormal EEGs, routine EEG is not a good screening tool. It should only be performed if there is clinical concern for seizures [83]. Even with routine EEG, making the diagnosis of epilepsy can be challenging. Therefore, concern for seizures warrants referral to a neurologist [80]. Often, video EEG monitoring is required to confirm the diagnosis of seizures [84]. To avoid unnecessary testing, caregivers of children with spells of staring and unresponsiveness should be instructed to provide stimulation through a loud sound, clap, or vigorous tactile stimulation. If the child responds, the events are unlikely to be seizure-related. Hand-waving has not been reported as a successful way to distract children from staring spells [69].

Metabolic and genetic evaluations are also important to consider in any child with intellectual disability and epilepsy. Metabolic evaluation is warranted if there is no identified

structural or syndromic cause of epilepsy [84]. However, inborn errors of metabolism are often associated with additional features, such as stroke and other neuroimaging abnormalities, and thus there should be a high index of suspicion for metabolic disorder in any infant who presents with seizures, even if a structural cause is identified [85]. A family history of epilepsy, myoclonic seizures, neuroregression, or encephalopathic episodes without structural or infective explanation should also trigger a metabolic evaluation [84]. Identification of additional neurologic and non-neurologic features are also suggestive of metabolic etiology [85]. Screening tests, including plasma amino acids, homocysteine, copper, ceruloplasmin, urine creatinine metabolites, glycosaminoglycans, oligosaccharides, urine organic acids, and pyrimidines/purines, identify approximately 60% of metabolic disorders metabolism [85].

Treatment of Seizures in Children with Neurodevelopmental Disabilities

In a child with a first unprovoked seizure, it is important to determine the likelihood of further seizures and, if so, what harm they could cause. This will help determine whether anti-seizure medications should be initiated. This is of particular importance for children with neurodevelopmental disabilities.

Medications are not always required after a first unprovoked seizure. There is no evidence to show remission of epilepsy is affected by waiting for a second seizure to initiate medication in most children [73]. Medications are associated with cognitive, behavioral, and systemic side effects. However, an underlying etiology, such as is found in the majority of children with neurodevelopmental disabilities, significantly increases the risk of seizure recurrence. In a meta-analysis, 32% of patients without a known cause experienced seizure recurrence after 2 years, compared to 57% with symptomatic etiology [86]. Furthermore, children with emerging epileptic encephalopathies require rapid initiation of treatment [84]. The use of medications in this population can be challenging, due to underlying comorbidities and polypharmacy, as well as increased likelihood of medically refractory epilepsy and status epilepticus.

The overall goals for treatment of epilepsy in children with neurodevelopmental disabilities are the same as the general population-seizure freedom, without medication effects. However, this is often not possible, as risk of intractability is higher [87]. Furthermore, intellectual outcome is worse and mortality rate higher in those with intractable epilepsy [1]. In a comprehensive Cochrane review, treatment with antiseizure medication reduced seizure frequency but did not result in higher rates of complete seizure freedom compared to placebo in patients with intellectual disability [88]. Therefore, the goals are often modified to maximize seizure control, minimize adverse effects, and improve quality of life.

Care must be taken when introducing medications in this population. First, consider how the treatment will affect the preexisting comorbidities. Children with impaired ambulation and coordination can decompensate with low doses of medications that cause dizziness, ataxia, or weight gain. They also may have more difficulty expressing symptoms of side effects, only demonstrating increased irritability or resisting activities [68]. Behavioral and cognitive effects of medications must be considered, especially in young children [89] (see Table 39.1 for cognitive side effects of medications). This must all be balanced against the impact of the seizures, including seizure severity and degree of interictal and ictal dysfunction [90].

Second, avoid over-medication, keeping in mind that these children are often already on other medications for treatment of comorbid conditions. Although seizures are more likely to be refractory, polypharmacy does not always yield improved seizure control but does often worsen side effects. A maximum of three anti-seizure medications has been recommended [68]. Monotherapy is the ideal goal, even in medically refractory. Quality of life often improves with medication reduction, even though seizure frequency is unchanged [68].

Third, if at all possible, consider withdrawal of medications in seizure-free patients. Children with a higher seizure burden (at least 10 seizures) before remission, multiple seizure types, remote symptomatic cause, developmental delay, abnormal neuroimaging, and persistently abnormal EEG are at increased risk for seizure recurrence [91]. Therefore, the risks of continued therapy must be carefully weighed with the potential harm due to seizure recurrence.

While it is important to avoid over-medication in this population and have reasonable expectations for seizure control, the risks of seizure-related morbidity and mortality are of concern to parents and cannot be ignored. Mortality rates in children with epilepsy have been reported to be 5 to 10 times higher compared to the general pediatric population. Furthermore, a meta-analysis of population and community-based studies of mortality in childhood epilepsy demonstrated the mortality rate in children with symptomatic epilepsy, such as those with neurodevelopmental disabilities, was more than 20 times higher, compared to children with uncomplicated epilepsy [92]. However, only 13/69 (19%) of the deaths were seizure-related. The remainder died of complications of their underlying disease [92]. Similarly, a 30-year population-based study of children with epilepsy demonstrated the overall mortality rate for children with epilepsy was 9 times higher than the national mortality rate for children. However, the epilepsy-related death rate, including SUDEP, was not significantly elevated [93]. Therefore, while

Table 39.1 Potential neurocognitive side effects of selected anti-seizure medications (Micromedex)

Medication	Sedation/Somnolence/fatigue	Insomnia	Decreased cognitive performance	Behavior change/irritability/psychiatric disturbance	Decreased coordination/ataxia/dizziness
Benzodiazepines (clobazam, clonazepam)	XXX	X		XX	X
Barbiturates (phenobarbital/primidone)	X	X	XXX	XXX	X
Carbamazepine	X				XX
Ethosuximide/methsuximide	X	X		X	X
Felbamate	XXX	XX			XX
Gabapentin	XX			X	XX
Lacosamide					XXX
Lamotrigine	XX	X			XX
Levetiracetam	XX			XXX	X
Oxcarbazepine/eslicarbazepine	XXX				XXX
Perampanel	XX			XX	XXX
Phenytoin	X	X	XX	X	XXX
Pregabalin	XXX			X	XXX
Rufinamide	XXX				XX
Topiramate	XXX		XXX	XX	XXX
Sodium valproate	XX				X
Vigabatrin	XXX		X	X	X
Zonisamide	XX		X	X	X

X = reported in <10% of patients
XX = reported 10–20% of patients
XXX = reported in >20% of patients

it is important to discuss seizure-related morbidity and mortality, it is also important to understand the impact of the child's neurodevelopmental disorder on long-term outcome.

Due to the high incidence of medically refractory epilepsy, children with developmental disabilities should be referred to tertiary or quaternary epilepsy care if seizure control has not been obtained after adequate trials of two medications in order to determine whether additional treatment options exist. If not previously completed and the epilepsy etiology remains unknown, a comprehensive metabolic and genetic evaluation should also be completed at this point. Treatment of the underlying metabolic disorder may improve seizure control. Furthermore, identification of a specific genetic or metabolic etiology may provide information on preferred treatment (see Tables 39.2 and 39.3). Other treatment options include dietary therapies, as well as resective or disconnective surgeries, corpus callosotomy, and neuromodulation.

The ketogenic diet (KD) is a potentially effective non-pharmacological treatment of intractable epilepsy at all ages. It provides a > 90% reduction in seizures in approximately 1/3 of patients, regardless of underlying etiology or seizure type [112]. Therefore, the KD should be offered to any child who is not seizure-free after trying two medications for whom other preferred treatments are not available [16]. The KD is particularly effective for some specific syndromes, such as myoclonic epilepsies, Dravet syndrome, and epilepsy with myoclonic atonic seizures (Doose syndrome), and is potentially helpful for other epileptic encephalopathies. Furthermore, it is the first-line therapy for two disorders of brain energy metabolism: GLUT1 deficiency syndrome and pyruvate dehydrogenase deficiency [16]. However, the KD can cause metabolic crisis in other metabolic disorders, such as disorders of fatty acid oxidation, pyruvate carboxylase deficiency, and some mitochondrial cytopathies. This further exemplifies the importance of a careful metabolic and genetic evaluation. Therefore, while the KD is potentially effective, initiation must be done under the guidance of an experienced ketogenic team [16]. The KD is likely of limited benefit for children who are potential epilepsy surgery candidates [16].

Children with intractable epilepsy should also be evaluated to determine whether they may be a candidate for epilepsy surgery. While there may be some concern that children with developmental disabilities may not receive the same benefit from surgery compared to normally developing children, this is not necessarily so. When children with intellectual disability (IQ ≤ 70) were compared to children with subaverage intelligence (IQ 71–85) and average intelligence (IQ > 85), 83% in the low-IQ were seizure-free, compared to 62% in the subaverage and 74% in the average intelligence groups at long-term follow-up [113]. A shorter duration of epilepsy has been associated with higher IQ scores, and the children with lower IQ had more cognitive improvement than those with higher presurgical IQ [114]. Epilepsy surgery also has the potential to allow a decrease in anti-seizure medications, which may have a beneficial effect on overall cognitive outcome [115]. Finally, health-related quality of life improvements following epilepsy surgery are similar for children with and without low intellectual ability [116].

For cases not amenable to resection, palliative surgical interventions could be considered. Patients with generalized drop attacks may benefit markedly from corpus callosotomy, with resolution or significant decrease in drop attacks reported in 54–90% of cases [17, 117]. Vagus nerve stimulation (VNS) is also approved as adjunctive therapy for epilepsy, with a greater than 50% seizure reduction in 55% of children with focal or generalized onset epilepsy [118]. Finally, intracranial stimulation, including cortical stimulation and thalamic deep brain stimulation, is an emerging therapeutic option for children with epilepsy who are not resection candidates [119, 120].

Table 39.2 Genetic disorders associated with specific epilepsy syndromes and developmental disabilities

Disorder	Neurodevelopmental disabilities	Features of epilepsy	Specific therapy
Down Syndrome (Trisomy 21) [94]	– Varying degrees of intellectual disability – Increased risk for early onset dementia	– Epileptic spasms are most commonly reported seizure type – Generalized epilepsies – Focal epilepsies	Spasm-specific therapies (ACTH, vigabatrin, prednisone). Reported better response to ACTH
Fragile X [95]	– Males typically only affected (female carriers can be symptomatic) – Intellectual disability – ADHD – FMRP can lead to neuronal hyperexcitability and increased seizure risk	– Focal seizures reported most commonly – Generalized seizures less common – EEG findings can mimic other childhood epilepsies, particularly BECTS)	No specific therapies. Majority have well-controlled seizures

Table 39.2 (continued)

Disorder		Neurodevelopmental disabilities	Features of epilepsy	Specific therapy
CDKL5 (cyclin-dependent-kinase like-5) [96, 97]		– Severe developmental delay – Visual impairment – Early onset epileptic encephalopathy	– Hypermotor-tonic-spasms sequence – Epileptic spasms – Focal seizures – Myoclonic seizures	Refractory epilepsy Treatments which may be beneficial include topiramate, vigabatrin, and ketogenic diet
ARX (Aristaless-related homeobox protein/Xp21.3) [96]		– Depending on variant can see different phenotypes including malformations of cortical development, severe intellectual disability, abnormal genitalia, hydrocephalus, movement disorder, and refractory early onset epilepsy/epileptic encephalopathy	– Epileptic spasms (typically before 6 months) – Tonic – Atonic – Myoclonic – Focal seizures	No specific therapy. Refractory epilepsy
Sodium channelopathies	SCN1A [98]	Dravet syndrome/ severe myoclonic epilepsy of infancy – Normal early development followed by loss of skills – Onset of recurrent febrile hemi-convulsive seizures in first year of life – Emergence of additional seizure types – Development of extrapyramidal movement disorder	– Febrile convulsive and hemiconvulsive generalized tonic-clonic seizures – Febrile status epilepticus – Myoclonic seizures – Generalized tonic-clonic – Atypical absence – Tonic – Atonic – Temperature-induced seizures – Pattern-induced seizures	Valproic acid and clobazam considered first line. Second line are stiripentol, topiramate, ketogenic diet. Cannabadiol can be beneficial. Investigational therapies include fenfluramine. Avoid sodium-blocking agents (carbamazepine, oxcarbazepine, lamotrigine, phenytoin)
	SCN2A [99]	Malignant migrating focal epilepsy of infancy – Early onset epilepsy – Severe intellectual disability – Movement disorder – Axial hypotonia – Appendicular spasticity	– Malignant migrating focal epilepsy of infancy – Ohtahara syndrome (early infantile epileptic encephalopathy) – Epileptic spasms	Can respond well to sodium-blocking agents (phenytoin)
PCDH19 (protocadherin-19) / Xq22 [100]		– Females with a Dravet-like phenotype – Normal early development with seizure onset and regression typically around first year – Febrile and temperature-induced seizures – Autistic-like features	– Febrile and afebrile GTCs – Febrile and afebrile status epilepticus – Focal seizures – Atypical absence (less frequent than in SCN1A) – Myoclonic seizures (less frequent than in SCN1A)	Can respond to similar medications used in Dravet (valproic acid, clobazam, stiripentol)
Tuberous sclerosis complex [50, 101]		– Wide phenotypic spectrum – Intellectual disability can range from mild to severe – Autism-spectrum disorder	– Epileptic spasms – Asymmetric tonic spasms – Focal seizures – Generalized tonic-clonic seizures	mTOR inhibitors such as everolimus, sirolimus, and partial inhibitor vigabatrin. Focal respective surgery for dominant tuber

Table 39.3 Metabolic disorders associated with specific epilepsy syndromes and neurodevelopmental disabilities

Disorder		Description	Specific therapy
Pyridoxine-responsive epilepsy [102, 103]	Pyridoxine-dependent epilepsy/Antiquitin deficiency (*ALDH7A1* gene)	– Classic: Refractory neonatal seizures responsive to intravenous pyridoxine – Late onset: >2 years with refractory seizures. Response to pyridoxine can be delayed compared to neonatal form – Seizure types include tonic, atonic, myoclonic, and focal – Intellectual disability and can have nonspecific brain abnormalities	Pyridoxine: An acute trial of 100 mg IV pyridoxine followed by oral maintenance course at 15–30 mg/kg/day if response seen
	Pyridox(am)ine 5′-phosphate oxidase deficiency/PNPO	– Neonatal or infantile onset epileptic encephalopathy – Earlier onset may respond to pyridoxine but epilepsy tends to be refractory to pyridoxine and antiseizure medications – Seizure types include generalized tonic-clonic, tonic, atonic, atypical absence, myoclonic, and focal – Can have systemic manifestations including intellectual disability, eye findings, movement disorder	Oral Pyridoxal-5-phosphate (PLP) 30–60 mg/kg/day. Some patients require higher dosing up to 100 mg/kg/day
Biotinidase deficiency [104]		– Refractory epilepsy with onset within first year of life – Seizure types can include epileptic spasms, generalized tonic-clonic, tonic, atypical absence, and focal – Systemic symptoms include hypotonia, ataxia, skin rash, alopecia, intellectual disability, optic neuropathy, hearing loss	Oral biotin supplementation (5–10 mg/day)
Creatine deficiency [105]		– Three forms: GAMT, AGAT, and CT1 – Seizures and intellectual disability most common symptoms with seizure onset within first year of life in GAMT – Seizures frequent in CT1 but not presenting symptoms and often respond to antiseizure medication – Multiple seizure types can be seen including febrile seizures, generalized tonic–clonic seizures, and myoclonic-atonic, tonic, atonic, atypical absence, and focal – Movement abnormalities including chorea, athetosis, ballismus and dystonia – Other symptoms seen can include autism and severe language delay	Creatine monohydrate (350 mg/kg/day to 2 g/kg/day). Dietary restriction of arginine (to 15 mg/kg/day). Ornithine supplementation (350–800 mg/kg/day)
Cerebral folate deficiency [104]		– Folinic responsive epilepsy – Initial normal development followed by regression autistic-like features, behavioral change movement disorder (ataxia, hypotonia, spasticity, dyskinesia), slowed head growth, and epilepsy during infant-toddler years – Myoclonic seizures most commonly reported. Also seen are tonic and atonic seizures, and spasms	Oral folinic acid (1–5 mg/kg/day)
Glucose transporter deficiency (GLUT1) [106]		– Majority secondary to SLC2A1 mutations – Classic form: early-onset epileptic encephalopathy, acquired microcephaly, developmental delay, hypotonia, spasticity, and movement disorders – Variants: early-onset absence epilepsy, paroxysmal dysknesia, dystonia, choreoathetosis, alternating hemiplegia, intermittent ataxia, language delay and learning disorders	Ketogenic diet

Mitochondrial disorders [107, 108]	MELAS (mitochondrial encephalomyelopathy, lactic acidosis, and stroke-like episodes)	– Recurrent stroke-like episodes associated with imaging changes that have complete or near complete resolution – Over time, deficits accumulate leading to progressive neurologic disability – Imaging and EEG abnormalities have a predisposition to occur in the occipital regions – Seizures most commonly are focal and multifocal onset and can be presenting symptom	Oral arginine supplementation recommended. IV arginine for acute crises. Additional supplement efficacy anecdotal Avoidance of mitochondrial toxic medications
	POLG1 (polymerase gamma)/Alpers-Huttenlocher syndrome	– Epilepsy onset can be sudden and refractory – Convulsive and non-convulsive status epilepticus, myoclonic status epilepticus, focal and multifocal seizures – Predilection for epileptiform abnormalities and seizure onset from occipital lobe	Epilepsy can be intractable to medication Valproic acid contraindicated as can lead to liver failure in these patients
	Leigh syndrome (subacute necrotizing encephalopathy)	– Genetic and phenotypic variability with onset ranging from neonatal to adult – Common symptoms include neurodevelopmental regression/delay, hypotonia, truncal ataxia, tremor, and lactic acidosis – Imaging demonstrates focal abnormalities in the hypothalamus, thalamus, basal ganglia, cerebellum, brainstem – Seizure types seen include epileptic spasms, focal and generalized	Some patients may benefit with supplementation of thiamine (B1), coenzyme-Q, and carnitine. Seizures may respond to ketogenic diet
Peroxisomal disorders [109]	Zellweger-spectrum disorders/PEX gene mutation	– Disorder of peroxisome biogenesis – Neonatal-onset epilepsy secondary to neuronal migration abnormalities, which tend to be localized to opercular region – Neonates tend to be hypotonic with poor feeding – Other symptoms include severe intellectual disability, renal cysts, adrenal dysfunction, chondrodysplasia punctata, liver disease, peripheral neuropathy, ataxia	Symptomatic treatment. No contraindications for antiseizure medication use
Lysosomal disorders [110, 111]	Neuronal ceroid lipofuscinoses (infantile and late infantile NCL/CLN2)	– Onset typically between 2 and 4 years with new onset epilepsy and/or ataxia – Majority have a history of expressive language delay – Course is progressive with developmental regression, movement disorder, vision loss, and – EEG can demonstrate photoparosymal response at low stimulation frequencies (1–3 Hz)	Intraventricular infusion of ceroliponase-alfa has been found to have reduced motor and language decline
	Gaucher disease/ beta-glucocerebrosidase deficiency	– Types 2 (acute/infantile-onset) and 3 (chronic) associated with epilepsy – Features include horizontal supranuclear gaze palsy, intellectual disability, neuromuscular involvement, speech and swallowing difficulties – Type 2 has earlier seizure onset and is typically rapidly fatal – Progressive myoclonic epilepsy most commonly seen – EEG most commonly will demonstrate generalized discharges and additional generalized seizure types (tonic-clonic, atonic, tonic, atypical absence)	Therapies can include enzyme replacement therapy and/or substrate reduction therapy which can slow clinical course. Myoclonic epilepsy does not respond to enzyme replacement therapy

Summary Points

1. Cerebral Palsy
   (a) Children with cerebral palsy are at increased risk for the development of epilepsy, with the highest risk seen in those with spastic quadriplegia.
   (b) There is variability in the underlying etiology for cerebral palsy and therefore the types of seizures seen can be heterogeneous.
   (c) Factors associated with increased risk for development of epilepsy in CP are low 10 minute Apgar score, intellectual disability, and spastic quadriplegia.
2. Autism Spectrum disorder:
   (a) The risk of autism in children with epilepsy ranges from 5 to 37%.
   (b) There is a broad range in severity of epilepsy in children with autism.
   (c) There is no clear evidence that autism itself is a significant predictor of epilepsy outcome.
3. Malformations of cortical development:
   (a) Malformations of cortical development are the most common cause of pharmaco-resistant focal epilepsy in children.
   (b) Malformations of cortical development are highly correlated with pharmaco-resistance and may be amenable to surgical resection.
4. Fetal Alcohol Spectrum Disorders:
   (a) The pathogenesis of epileptogenesis in fetal alcohol spectrum disorders is likely multifactorial, with both direct toxic effects of ethanol on the developing brain as well as disruption of normal cellular function.
   (b) The risk for the development of epilepsy in fetal alcohol spectrum disorders is increased with early and sustained alcohol use during pregnancy.
5. Investigation of seizures in children with neurodevelopmental disabilities:
   (a) History and physical exam are essential to the evaluation of a child with seizures in order to determine how urgently treatment is required, determine risk of seizure recurrence, and identify underlying etiologies.
   (b) EEG is recommended as standard of practice for evaluating epilepsy. However, routine EEG may be of limited value in children with ASD due to high incidence of abnormal EEGs in ASD children without epilepsy and prolonged video EEG may be required.
   (c) Metabolic and genetic evaluations can be considered in all children with intellectual disability and epilepsy.
6. Treatment of seizures in children with neurodevelopmental disabilities:
   (a) Medications are not always required after a first unprovoked seizure, although children with developmental disabilities are more likely to experience seizure recurrence than children without disabilities.
   (b) Children with developmental disabilities are more likely to have intractable epilepsy. Seizure control and medication side effects must be carefully balanced.
   (c) Additional treatment options include ketogenic diet, surgery, and neuromodulation and are typically used with medications.

Clinical Vignettes

1. A 2.5-year-old girl presented with recurrent clusters of sudden arm extension and abduction with truncal flexion occurring multiple times per day. Her parents also noticed a decline in her development, especially language. Her EEG showed these events were consistent with epileptic spasms. Furthermore, the EEG demonstrated frequent generalized epileptiform discharges during and in between seizures, as well as significant slowing of the background activity. However, the EEG discharge with some of the events was asymmetric and the medical team noted she reliably turned to one side during the seizures. Her MRI demonstrated a lesion over the mesial right temporal lobe that was hypointense on T1 sequences (Fig. 39.1a) and hyperintense on T2 sequences (Fig. 39.1b). Multiple medication trials were unsuccessful at controlling seizures and the child continued to experience developmental regression.

Further evaluation demonstrated the seizures were likely coming from the area of the MRI abnormality, even though the majority of the EEG abnormalities were generalized. She underwent resection of the lesion and the cortex surrounding it. Pathology revealed ganglioglioma (WHO grade 1).

Following resection, there was complete resolution of seizures. All potentially epileptiform activity resolved and the background activity normalized. Developmental milestones were regained. She continued to have a language-based learning disorder, but was within normal limits for all other cognitive domains. She was subsequently able to wean off all anti-seizure medications.

2. This 4-year-old, left-hand dominant girl presented with a three-year history of intractable seizures which consisted of nausea, staring, and pallor, followed by rightward head deviation and right-sided stiffening. These occurred multiple times per day, and had been refractory to four antiseizure medications. She was born at 35 weeks gestation and at a week of age-developed lethargy and seizures and was diagnosed as post-hemorrhagic hydrocephalus requiring a ventriculoperitoneal shunt. Seizures persisted postoperatively, and an MRI then showed sinovenous thrombosis of the posterior sagittal sinus and the left transverse sinus, with left basal ganglia ischemia. On examination, she had

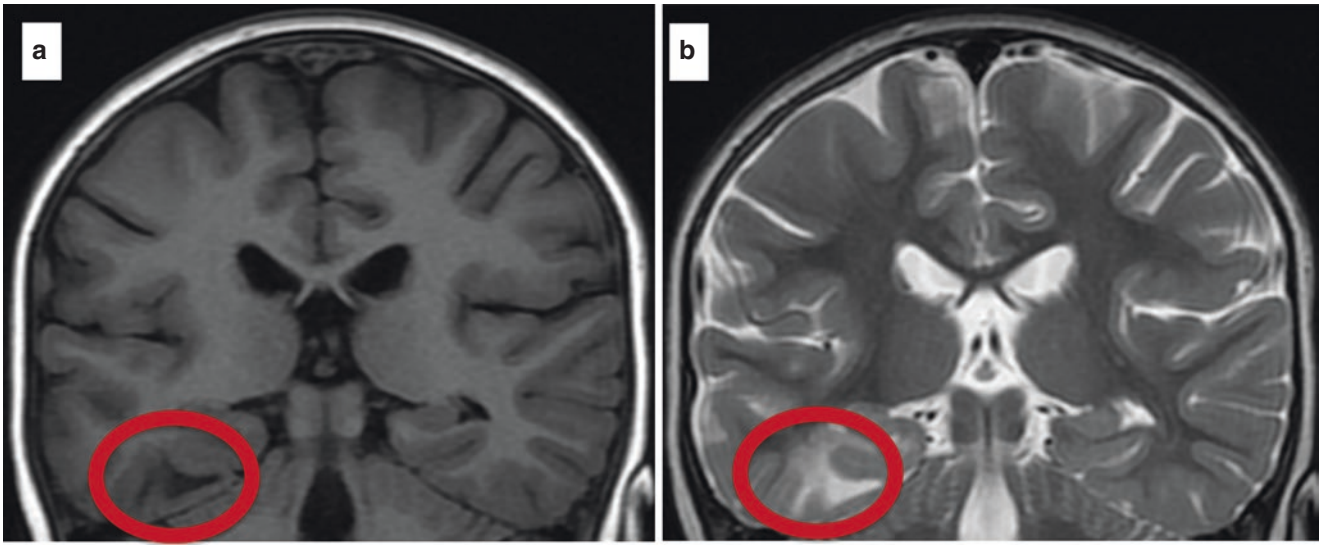

Fig. 39.1 Coronal MRI images demonstrating right mesial temporal lesion that was hypointense on T1 sequences (**a**) and hyperintense on T2 sequences (**b**)

a right hemiparesis. Neuropsychometric testing showed her verbal and nonverbal skills to be in the average range but she had difficulties with impulsivity and poor attention.

Her EEG showed frequent left and midline frontal discharges, as well as generalized discharge, with left frontal predominance. In sleep, discharges became near continuous, and spread to the right hemisphere as well (Fig. 39.2a). Her MRI showed moderate volume loss in the left hemisphere with a left frontal ventriculoperitoneal shunt. She was admitted to the pediatric epilepsy monitoring unit and numerous clinical and electrographic seizures were recorded, with diffuse left hemispheric onset.

She underwent a functional hemispherotomy with resolution of seizures, and improvement in her focus and attention. At follow-up three years later, she remains seizure-free, and is functioning at a grade appropriate level. Her follow-up EEG continues to show frequent discharges and slowing in the disconnected left hemisphere, but no independent discharges in the right hemisphere, which now shows normal spindles in sleep (Fig. 39.2b).

3. A 5-month-old previously healthy boy presented to the emergency department with a history of two weeks of recurrent daily paroxysms characterized by truncal flexion with bilateral arm abduction. He was diagnosed with epileptic spasms and brain MRI demonstrated multiple cortical tubers, consistent with tuberous sclerosis. Spasms were initially refractory to vigabatrin and then ketogenic diet, but eventually he became spasm-free on a combination of vigabatrin and topiramate. Development had been normal until onset of spasms, after which time, milestones were obtained more slowly.

Around age 2 years, he developed focal onset seizures characterized by behavioral arrest, slurred speech, decreased interaction, and unsteady gait. On overnight EEG recordings, subclinical seizures were recorded arising from the left frontocentrotemporal head regions and clinical seizures were seen arising from the left frontocentral region.

At age 3 years he continued to have frequent focal seizures as well as prolonged periods of reduced responsiveness concerning for subtle seizures. He underwent epilepsy surgery evaluation with implantation of grid and strip electrodes for intracranial EEG monitoring. Seizures were observed arising from the left frontal grid in the region of a partially calcified cortical tuber (Fig. 39.3a). He subsequently underwent left frontal tuber resection (Fig. 39.3b). On most recent follow-up at age 9 years, he was demonstrating issues with impulsivity and emotional disinhibition, with improved seizure control.

4. This 11-year-old boy, with a history of focal impaired awareness seizures, developmental delay and autism since early childhood presented with a three-month history of regression in his skills. He was less interactive, had regressed in speech and academic skills, and now needed assistance to feed and dress himself. When spoken to, he would respond with single words at best but attempted to follow commands. He had an action tremor and cogwheeling of both upper extremities and his gait was bradykinetic gait, with decreased arm swing. His EEG showed diffuse background slowing with multifocal epileptiform discharges.

An MRI spectroscopy showed a markedly attenuated creatine peak (Fig. 39.4). He was subsequently diagnosed with creatine transporter deficiency and showed improvement following high dose creatine supplementation.

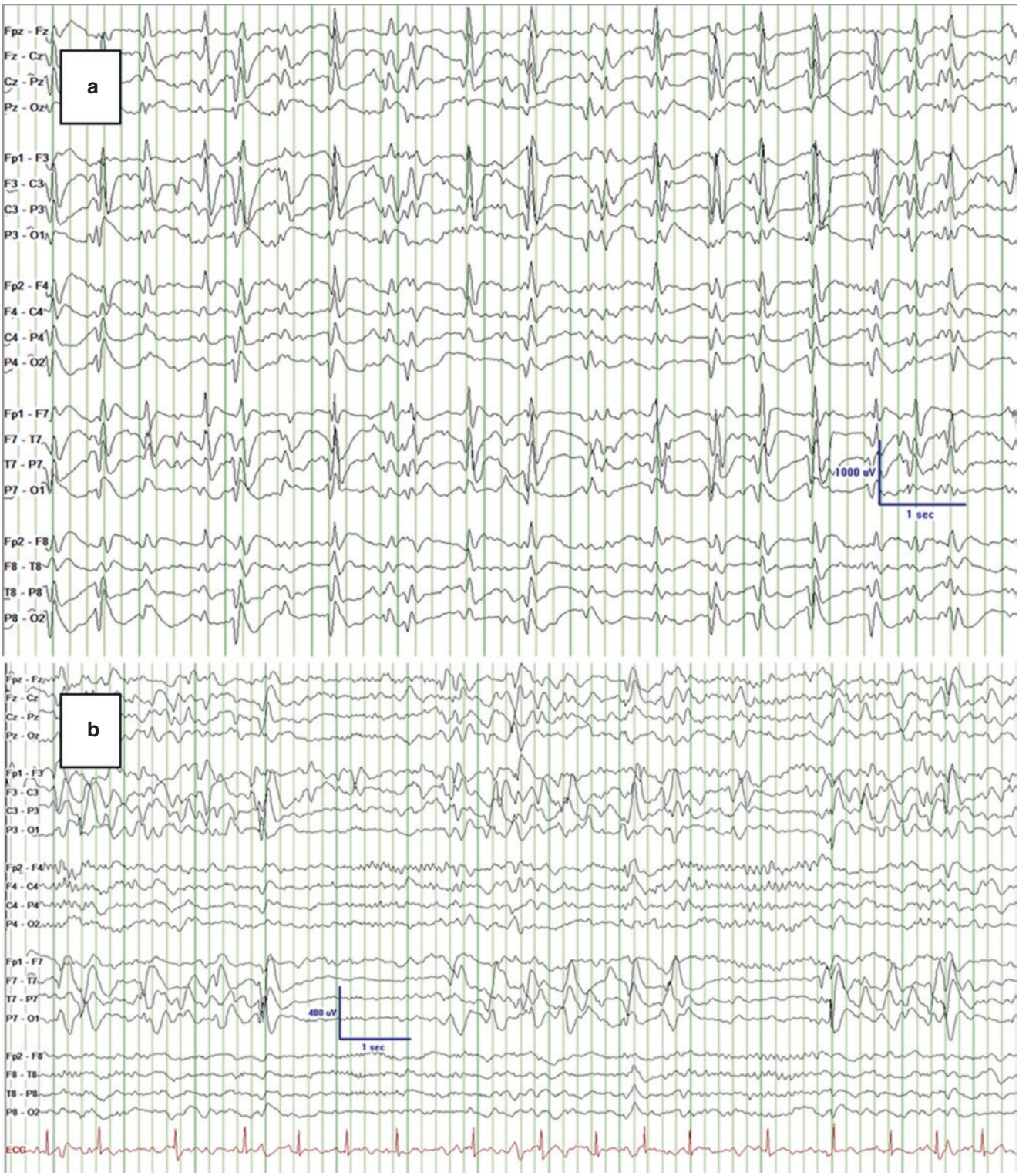

Fig. 39.2 (**a**) EEG showing continuous spike wave discharge in sleep. Note higher amplitude over the left hemisphere. (**b**) EEG following left hemispherotomy showing resolution of continuous spike wave discharges in the right hemisphere, with normal sleep spindles. Discharges and slowing persist in the disconnected left hemisphere

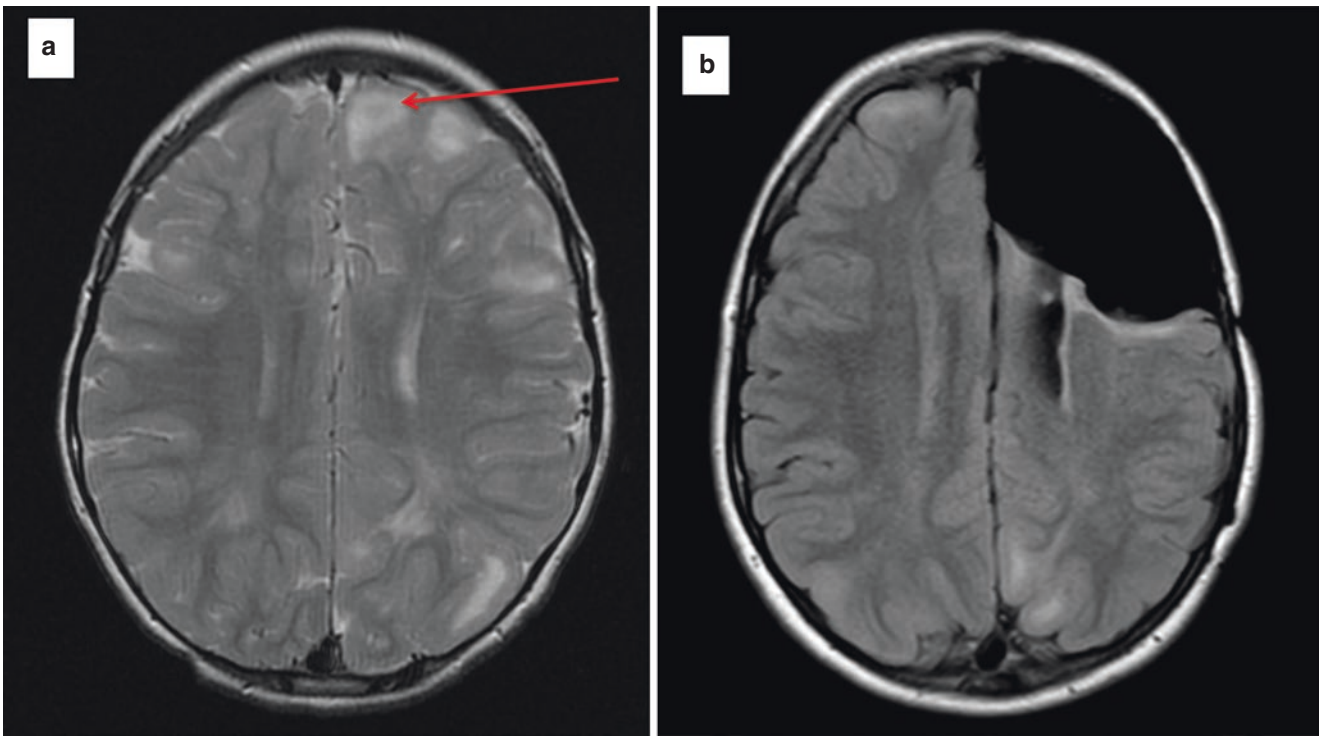

Fig. 39.3 (**a**) Axial T2 MRI image demonstrating multiple cortical tubers in a patient with Tuberous Sclerosis and a large partially calcified left frontal tuber (arrow). (**b**) Axial T2 FLAIR MRI image of the same patient following left frontal tuber resection

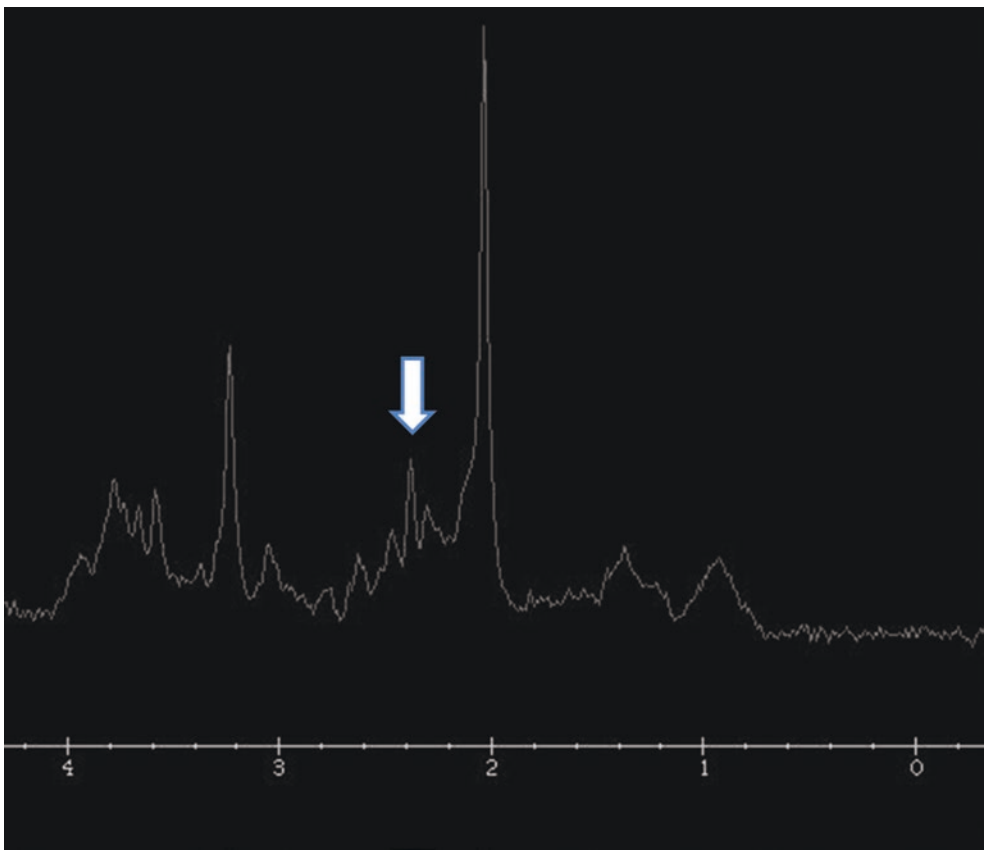

Fig. 39.4 MRI spectroscopy showing markedly attenuated creatine peak (arrow)

5. A 3-year-old girl with known Angelman syndrome and subsequent epilepsy returned to neurology for staring spells. EEG recorded during the staring spells showed no EEG change during them. Furthermore, parents were able to get her attention with tactile stimulation. However, the neurologist was concerned that she was having frequent subclinical seizures due to prolonged trains of potentially epileptiform discharges. She was referred for an epilepsy subspecialty consult. EEG demonstrated frequent diffuse slow waves with a sharply contoured notch associated (Fig. 39.5a, b).

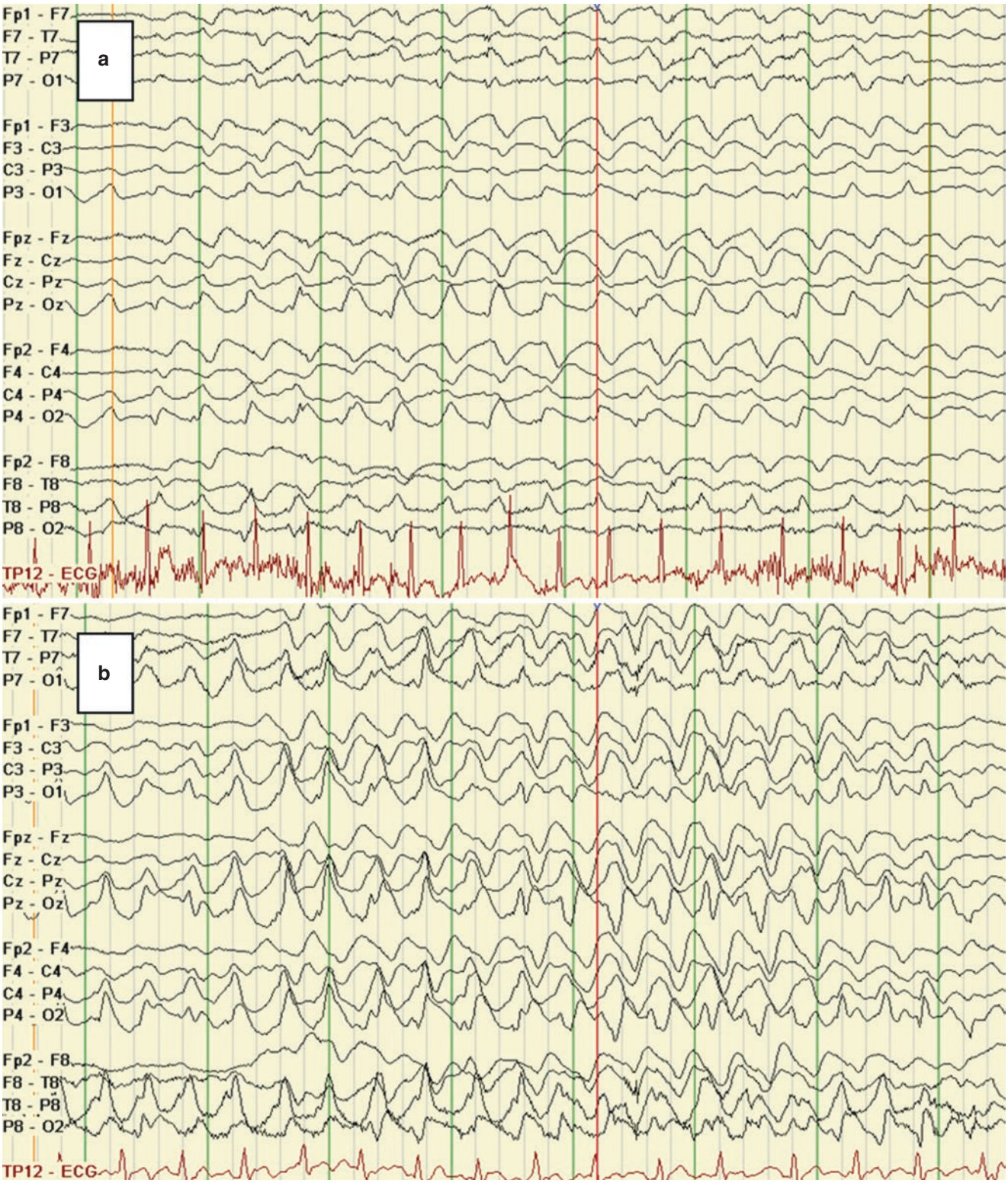

Fig. 39.5 (**a**, **b**) EEG demonstrating frequent diffuse slow waves with a sharply contoured notch, consistent with notched delta

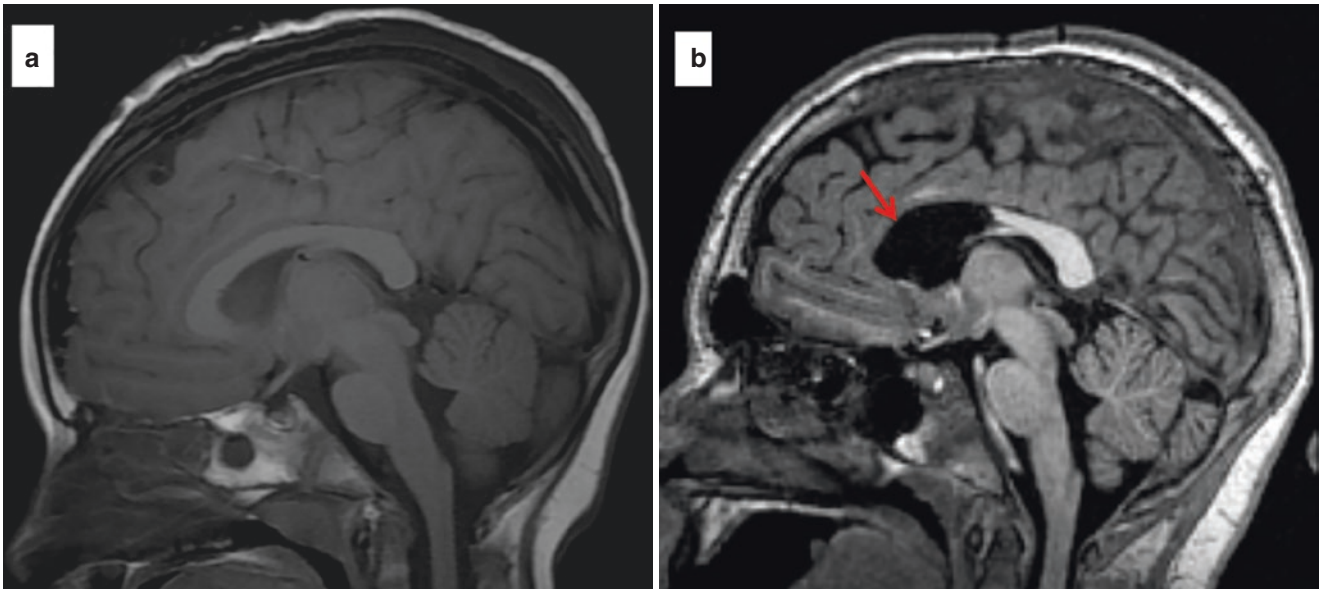

Fig. 39.6 Sagittal T1 MRI images of the patient before (**a**) and after (**b**) anterior two-thirds corpus callosotomy (arrow)

The EEG was consistent with notched delta, which is a common inter-ictal finding in patients with Angelman syndrome. The EEG was not consistent with subclinical seizures.

6. A 12-year-old girl with Lennox–Gastaut syndrome secondary to diffuse polymicrogyria was evaluated for epilepsy surgery. She had multiple seizure semiologies including frequent tonic, generalized tonic-clonic, focal motor, atypical absence, and myoclonic seizures that were refractory to multiple antiseizure medications. Her tonic seizures occurred mainly in the early morning hours and if she was standing, she would frequently become injured with these. At baseline, she was verbal and ambulatory; however, parents noted slowing of developmental progress after 5 years of age. Given her frequent tonic seizures resulting in injury, an anterior two-thirds corpus callosotomy was recommended given her current level of functioning. She underwent an uncomplicated anterior corpus callosotomy (Fig. 39.6a, b) which resulted in significant reduction in tonic seizures from multiple daily to one to two per week, as well as reduction in her generalized tonic-clonic seizures from weekly to several per year.

Multiple Choice Questions

1. Which is the MOST significant risk factor for the development of epilepsy in a child with ASD?
   (a) Older age
   (b) Male gender
   (c) Severity of intellectual disability
   (d) Regression of skills
   Answer: (c)
2. Which of the following antiseizure medications are LEAST likely to exacerbate problematic behaviors?
   (a) Clobazam
   (b) Topiramate
   (c) Perampanel
   (d) Levetiracetam
   (e) Phenobarbital
   Answer: (b)
3. In children with epilepsy and CP which of the following is true?
   (a) Certain etiologies of CP are more often associated with epilepsy
   (b) Children with CP always have neonatal onset seizures
   (c) Epilepsy development has no relationship with motor impairment
   (d) Epilepsy in CP is always medically refractory
   Answer: (a)
4. A child with a history of CP presents to the emergency department for evaluation of a possible first time seizure. The child was not ill and is currently at her baseline. Which of the following investigations must be completed in the emergency department?
   (a) EEG
   (b) CT of head
   (c) History and physical exam
   (d) Complete blood count and electrolytes
   (e) All of the above
   Answer: (c)

5. Epilepsy treatment in children with developmental disabilities includes
   (a) Balancing seizure control with medication side effects, recognizing a high risk of medical intractability
   (b) Medication monotherapy, if possible
   (c) Dietary therapies, surgery, and neuromodulation
   (d) A and B
   (e) A, B, and C

   Answer: (e)

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Behavioral and Mental Health Disorders (Including Attentional Disorders)

40

I. Leslie Rubin, Claire D. Coles, and Jarrett Barnhill

Learning Objectives

Upon completion of this section, the reader will be able to

- Demonstrate an understanding of the multidirectional interrelationship between these disorders and ecological/environmental influences.
- Apply the principles to the differential diagnosis of cerebral palsy and fetal alcohol spectrum.
- Discuss how ASD, FASD, CP ADHD phenotypes relate to mental health disorders.
- Discuss basic treatment concepts for the complex disorders.

Highlights

- CP, FASD, ASD, and ADHD lie on a spectrum of heterogeneous neurodevelopmental disorders that contribute too many neurobiological and behavioral phenotypes.
- The high rates of co-occurrence and comorbidity between these disorders mean that even a relatively straightforward syndrome like ADHD can be a treatment challenge for some children.
- These behavioral and mental health disorders represent final common pathways of converging pathophysiologies.
- In spite of the revolution in the translational neurosciences, clinicians still struggle to make accurate diagnoses and effective treatment plans.
- Problems with both diagnosis and treatment relate to the transactional effects of adverse life events and other environmental/ecological events operating via complex epigenetic and neurobiological mechanism to create diagnostic and treatment problems.

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Introduction and Overview

Behavior encompasses the way an individual acts or conducts oneself towards self and others [1]. Behavior represents a dynamic interaction between an individual, the environment, and the people in it. An ecological approach best captures the transactional nature of child development by looking at a systems approach to human behavior [2].

Behavior disorders include patterns of behavior that contribute to functional impairments across multiple domains. MentalHealth.gov states: *Behavioral disorders involve a pattern of disruptive behaviors in children that last for at least 6 months and cause problems in school, at home and in social*

D. D. Eisenstat et al. (eds.), *Neurodevelopmental Pediatrics*, https://doi.org/10.1007/978-3-031-20792-1_40

situations [3]. The spectrum of problem behavior ranges from internalizing (anxiety, depression, inattention, social withdrawal) to externalizing disorders (include hyperactivity, disruptive, aggressive and destructive behaviors elements.

The National Alliance on Mental Illness defines mental illness as *a condition that affects a person's thinking, feeling, or mood* and ability [4] to relate to others. The diagnosis of a mental disorder requires meeting *specific* criteria that include significant levels of functional impairment, duration of symptoms, and specific exclusion criteria symptoms are Diagnostic and statistical manual of mental disorders, 5th edition (DSM-5).

Lastly, we need to understand the differences between mental illness and mental health. The CDC definition defines mental health as a sense of emotional, psychological, and social well-being, as well as how we think, feel and adapt, handle stress, relate to others, and make healthy choices [5].

Adverse Childhood Experiences

Adverse childhood experiences (ACEs) are a set of experiential and ecological events that can derail typical development, the child's growth and social/emotional development, that and contribute to chronic maladaptive behavior and mental illness (Fig. 40.1 see Chap. 50). The CDC uses the following list to capture the basic issues in mental illness [6]:

- Early adverse life experiences, such as trauma or a history of abuse (e.g., child abuse, sexual assault, and witnessing violence).
- Experiences related to other ongoing (chronic) medical condition.
- Biological factors, such as genes or chemical imbalances in the brain.
- Use of alcohol or recreational drugs.
- Having few friends.

Primary prevention of ACEs is our best intervention strategy. In a study of early childhood, psychological, physical, and sexual abuse requires usually occur in the context of family dysfunction, substance abuse, and mental illness. Embedded in this matrix is a spectrum of adverse events. These include domestic violence and a feeling of insecurity and lack of safety. Frequently, these adverse events result in a multi-generational dynamic that functions as a setting event in the precipitation and perpetuation of ACEs. For example, a study by the CDC and Kaiser Permanente surveyed a cohort of over 9000 individuals. The results suggested a direct correlation between childhood adverse experiences and later behavioral, physical and mental health problems, and early death [5, 7]. The behavioral and mental health problems included substance abuse, multiple sexual partners, and depression with suicide attempts. The negative health consequences included obesity, diabetes, heart disease, lung disease, skeletal fractures, liver disease, and stroke.

The intergeneration perpetuation of ACEs is particularly troublesome (Fig. 40.2). Chronic stress related to ACEs can result in an increase in the rate of premature births, intrauterine exposure related to substances of abuse, learning disabilities, ADHD, school failure, and other disabilities including cerebral palsy. ACEs also contribute to multigenerational psychiatric disorders such as post-traumatic stress disorder (PTSD). One mechanism, epigenetic effects on gene regulation, suggests a more complex bio-psycho-sociol etiology

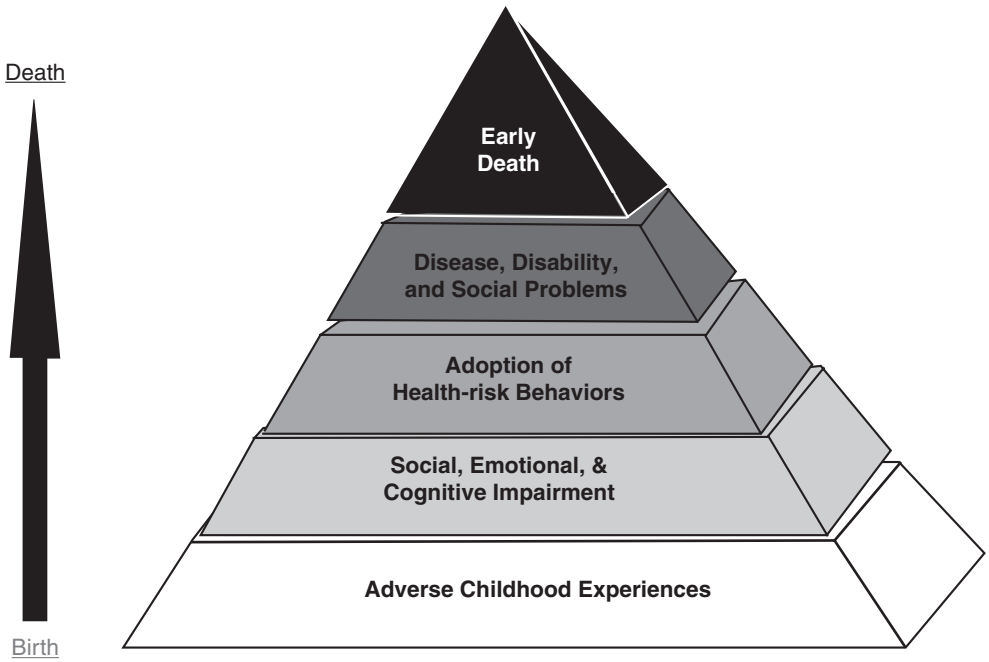

Fig. 40.1 Potential influences throughout the lifespan of adverse childhood experiences [5–7]

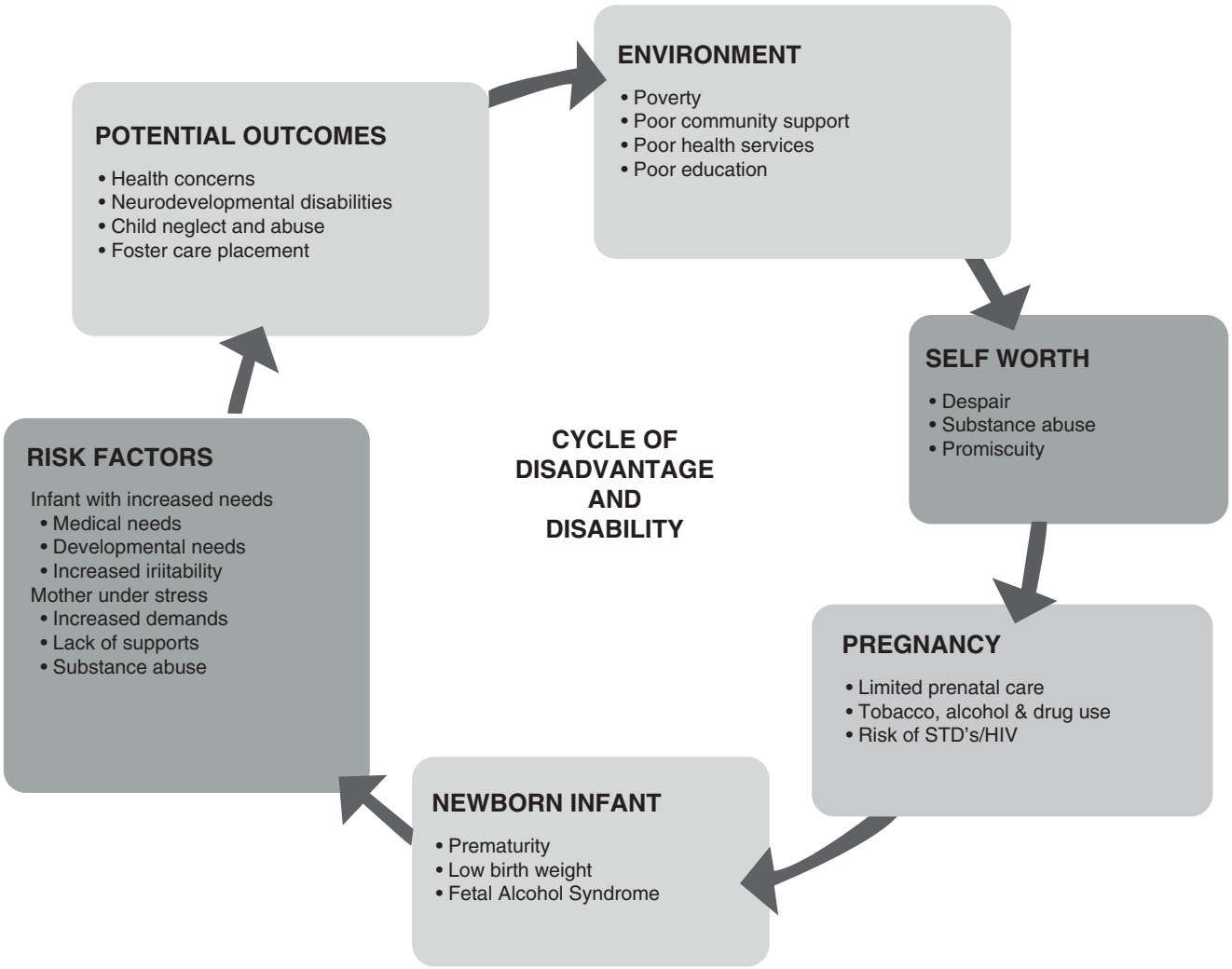

Fig. 40.2 Intergenerational cycle of social and economic disadvantage and neurodevelopmental disabilities in children [8, 9]

than previously considered. For our purposes, these adverse experiences remind us that the biology, culture and ecology of ACEs create and perpetuate the cycle of social and economic disadvantage, social dysfunction, and risk for neurodevelopmental disabilities [8].

Behavioral and Mental Health Disorders in Children with Neurodevelopmental Disorders

This chapter focuses on four specific neurodevelopmental disorders: attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (ASD), fetal alcohol spectrum disorder (FASD), and cerebral palsy (CP) [10]. We define FASD based on etiology:

1. Exposure to alcohol during critical periods of intrauterine growth and development,
2. Differential effects on sequential gene expression and functional gene modifications (epigenetic changes)
3. Adverse effects on developing neuronal cyto-architecture, synaptogenesis, developmental of white matter tracts, and emerging neurotransmitter networks.

CP represents a group of disorders affecting sensorimotor development that arise prenatally, perinatally, and postnatally. There are multiple factors associated with CP including genetic and environmental risk factors. The combination of deficits impact brain development and maturation and contributes to a range of neurocognitive, behavioral, and psychosocial outcomes.

ADHD is a complex polygenic neurodevelopmental disorder that affects many neuronal networks associated with emerging executive functions and other disorders.

Autism Spectrum Disorders

ASD is a complex polygenic disorder that derails the typical developmental trajectory of social-emotional development, social communication, interaction, and socialization. There may be two subtypes of ASD. One is secondary to the causes of underlying intellectual and developmental dIsabilities (IDD), neurobiological, or genetic/metabolic disorders. The other subtype relates to the concept of an expanded phenotype, is polygenic in nature (with high heritability), and the family frequently presents with more than one child with ASD [6, 11]. The behavior and mental health aspects of ASD are usually secondary to the pervasive developmental disorders observed in ASD.

The following considerations follow the DSM-5 diagnostic formulation [10], rearranged to help illustrate the associated behavioral and mental health manifestations.

Persistent Deficits in Social Communication and Social Interaction Across Multiple Contexts

Speech and Language

Social communication is cardinal among the diagnostic criteria for ASD. Early on, parental concerns about delays in speech acquisition raise the index of suspicion for ASD. Speech development is fundamental to communication, interaction, and socialization. Reciprocity, prosody, and pragmatics of speech and language also underlie emotional attachment and social interaction. If a child unable to communicate, he/she lacks major tools for developing self-control, expanding communication skills, learning, and managing frustrations. These deficits put the child at a disadvantage in terms affect regulation when frustrated and prone to emotional outbursts, anger and rage, tantrums, and various forms of aggression. Until understood as a communication deficits, caregivers and teachers misattribute these outbursts to primary "behavior problems."

An understanding and appreciation of this situation can help to mitigate the "behavior" or even prevent it. The keys to this approach are to focus on enhancing available modes of social communication, improving speech and communication through therapies or applying alternative modes of communication which may require personalized and appropriate assistive communication technology. To maximize effectiveness, the augmentation of therapies with behavior management strategies will enhance primary social skills and problem-solving strategies. Teaching anticipation and prevention strategies is useful in teaching across many settings as strategies to prevent and manage many problem behaviors [10].

Social Interaction

Difficulties with social integration are cardinal diagnostic criteria for the diagnosis of children ASD and can lead to behavior problems interacting with other people individually or in groups. The child may have difficulty reading social cues, nuances of gestures and social communication, or reciprocity in relationships or difficulties respecting personal space or social cues. Most social encounters are dynamic and rapidly changing. The child may lack the facility and adaptability needed to successfully master social situations. Not understanding the unspoken rules of interactions of social interaction, the child may respond with inappropriate responses and behaviors [10].

Restricted, Repetitive Patterns of Behavior, Interests, or Activities

Repetitive behaviors

In this context, children that may exhibit unusual repetitive behaviors are socially and contextually inappropriate. In addition, a common presentation includes an insistence on sameness, intolerance of deviation or change, and a rigid maintenance of restricted interests. These interests include rigidity of ideas, beliefs, and interests. The idee fixe may be frequently misattributed to symptoms of obsessive-compulsive disorder (OCD). In OCD, anxiety, over-intrusive thoughts or urges drive the compulsion. For many children with ASD, the repetitive behaviors frequently do not appear goal directed. Interrupting or preventing the child's rituals may contribute to frustration-induced tantrums (meltdowns), rage/aggressive behaviors, destructive behaviors, and self-injury. Alternatively, children may experience intense anxiety and distress and withdraw or disengage in response to changes, break in routines, or novel social settings [10].

Unusual Sensory Reactions

Overreaction to some stimuli such as loud sounds or crowds may result in unexpected behaviors. People in the environment who are not familiar with the child's sensitivities may wonder why suddenly this child is becoming distressed. The behavioral reaction may be misinterpreted as purely oppositional or stubbornness and evoke negative judgements from those unfamiliar with the characteristics of children with ASD.

Children with ASD are five times more likely to have mealtime challenges such as tantrums, extreme food selectivity, and ritualistic eating behaviors [12]. Although the feeding and eating patterns may be obsessively unusual and selective, resulting in concern for parents, consultation with a nutritionist to assure adequate nutritional intake, consulta-

tion with feeding therapists if the eating disorder is severe, and close monitoring of weight and height will assure parents of their child's health and growth.

Tendency to Overreaction

For multiple reasons seen above, children with ASD have difficulty in adapting an accommodating to changes, can easily be frustrated, and have poor self-regulation so they have a tendency to dramatic emotional reactions out of proportion to the of the situation [13]. These children may experience significant distress, withdraw, or have autonomic over arousal reactions. These overreactions can quickly escalate into tantrums, panic or rage-like episodes, with attempts to escape, destructive behavior, aggression, or self-injury (fright, flight-fight reactions spectrum [10, 13].

Parents and other providers can reduce high levels of anxiety-driven behaviors by identifying and modifying setting events or definable precursors. Likewise, other techniques that enable the child to modulate his or her responses to the stimulus will reduce the likelihood of decompensation. The approach to management and prevention is through improving self-regulation with a variety of therapies that include occupational therapy with a focus on sensory processing and sensory integration as well as aquatic therapy (hydrotherapy), hippotherapy (equine therapy) and other sensory-motor physical activities. Although sensory-based therapy remains to be firmly validated as being effective [14], it is supported and recognized as being effective by the CDC [15]. In addition, applied behavior analysis (ABA) focuses learning models designed to enhance attention, eye-contact/communication and skill development. The goals of therapy are to improve the ability of the child to learn new behaviors and improve adaptive skills. Variations in ABA expand the participation and focus for parents, teachers, and others playing active roles in these programs [16].

Psychiatric Comorbidities

The syndromal characteristics of ASD frequently overlap sensory and self-regulatory elements noted in behavioral and psychiatric disorders. Recognition of the linkages is frequently related to the level of associated with comorbid neurodevelopmental disorders (e.g., IDD) and the nature of these skill deficits. Likewise children with ASD are more likely to have ADHD, anxiety, OCD, sleep disorders, Tics and Tourette Syndrome, depression, bipolar disorder, episodic dyscontrol syndrome, psychoses, and catatonia. The severity of these comorbid conditions can overshadow the core features of ASD, and the therapies shift to those designed for primary psychiatric disorders. This shift frequently results in more restrictive placements, and more intense medical, behavioral, and pharmacological interventions [17, 18].

Given the unusual behavior characteristics of children with ASD, it is not surprising that, historically, professionals labeled these "odd" children with childhood schizophrenia. The work of Kanner and the British Working Group challenged this assertion, citing major clinical differences between these infantile autism (ASD) and childhood schizophrenia. In recent years, interest in the same boundary issues is spilling over into genetic markers, neurobiology, neuroimaging, and broader investigations into their developmental trajectories [19].

Anxiety

Among adolescent with ASD, many assessments focus on mixtures of mood and anxiety. These presenting symptoms may be misattributed to anxiety disorders [20]. Clinicians seem sometimes forget that anxiety and mood disorders are among the most common psychiatric disorders in children and adolescence. A similar trend appears in children with ASD. Anxiety as an affective state is not a core feature of ASD but accompanies deficits in social communication and uncertainty that may increase repetitive-restrictive interests and behaviors. Many social interactions and life circumstances appear anxiogenic to internalizing children [21].

These same forces affect children's vulnerable to anxiety and mood disorders. In many cases, the boundaries between these two affective disorders are fluid during childhood. Behavioral manifestations rather than affective changes overshadow mood and affective symptoms in post-pubertal females. Additionally, the older adolescence experiences the burden of social isolation and a sense of being unable to fit in. These social shortcomings contribute to loneliness and life-dissatisfaction for many higher functioning adolescences with ASD. Demoralization and fatalism over difficult life transitions play a major role in mood disorders in early adulthood [22].

Tics and Tourette's

The prevalence of tic disorders among children with ASD is probably higher than current estimates. This may be due to difficulties with recognition of secondary to boundary issues between complex tics, and OCD or related symptoms. Weaknesses in social and communication skills in Tourette Syndrome appear similar those observed in ASD. The problem becomes even more challenging when childhood-onset tics wane or gradually resolve by late adolescence [23].

ADHD

Core symptoms of ADHD (attention deficit, impulsivity, and hyperactivity) are part of the clinical picture of ASD [24]. and will be discussed later in this chapter.

Medical Conditions Expressing as Behavior Disorders

Because children with ASD have limited communication skills and variable pain thresholds, emotional and behavioral response tend to overshadow pain and common medical problems. For example, gastrointestinal disorders such as gastroesophageal reflux and constipation may generate

disruptive behaviors. These behavioral modes of expressing discomfort can be ambiguous or inadequately communicated. Even acute, nociceptive pain such as headache, dental pain, or acute abdominal pain go undetected. Because of this, physicians and other healthcare providers should maintain a low threshold of suspicion and remain vigilant for medical causes when presenting behaviors seem inconsistent with obvious environemntal of emotional stressors [25].

Disparities in Diagnosis and Treatment of ASD

In their 2018 morbidity and mortality weekly report (MMWR), the series is prepared by the centers for disease control and prevention (CDC) reported significant disparities between prevalence rates of ASD across 11 autism centers around the United States. The prevalence in these locations varied from 16.8 cases/1000 children to as high as nearly 2/100 (1 in 59). Male: female ratios of 4 to 1 remained steady even though many females still go unrecognized [26]. These prevalence rates vary considerably from state to state and the distance from academic centers specializing in ASD services. Furthermore, the CDC reported socioeconomic, ethnic, and racial disparities reflected in varying prevalence rates among whites that were 7% greater than that among black children and 22% greater than Latinx children were. The consensus derived from these reports suggests that ethnic and racial disparities across geographic regions can impact early diagnosis [24, 26–30].

These delays in recognition and diagnosis significantly influence access to early intervention, treatment resources. This is unfortunate since early interventions have a greater positive effect on subsequent development than later interventions. Irrespective of IQ scores, early therapeutic interventions are likely to have a protective effect on the evolution of behavioral and psychiatric disorders [29]. It is critically important to compensate for these disparities by improving services to underserved children [30].

Section Bullets

- Child developmental is complex process that unfolds over the developmental period.
- There are critical periods during prenatal, perinatal, postnatal developmental insults that have a disproportionate impact on gene regulation and brain development and maturation.
- ACEs play a key role on atypical patterns of cognitive, emotional, cognitive and social development.
- Adverse social events negatively affect health, and well-being throughout the lifespan.
- ASD is a complex neurodevelopmental disorder that affects emerging social communication and mental/behavioral flexibility and adaptability.
- ASD is a heterogeneous disorder that includes a familial form (expanded phenotype), and a "secondary form that occurs in many genetic disorders.
- The severity of co-occurring IDD ads to this complexity.
- ASD is frequently comorbid with multiple mental health disorders.
- Recognition and diagnosis are challenging, especially since many core aspects of ASD overshadow symptoms of mental disorders.

Cerebral Palsy

Children with CP are more likely to exhibit other neurodevelopmental disorders as well as behavior and mental health disorders than the general population. CP is a disorder of movement and posture that represents a fixed insult to the developing brain before, during or soon after birth. The diagnosis of CP is based on the presence on motor characteristics with significant functional implications, but reality is that the central nervous system (CNS) lesions are more often than not, diffuse and involve other functional elements. Only in a few cases of CP such as a middle cerebral artery infarction resulting in a hemiplegia, or a lesion in the basal ganglia resulting in an extrapyramidal picture, are there unlikely to be other functional implications. Other etiologies are less focused and specific, resulting in other neurodevelopmental consequences (see Fig. 40.3). The CDC reports that more than 40% of children with CP had intellectual disability, 35% had epilepsy, and more than 15% had vision impairment. Nearly 1 in 4 children with CP had both intellectual disability and epilepsy [31]. They also report a prevalence of ASD of 6.9%, which is much greater than the general population. Interestingly they found that co-occurring ASD frequency was higher among children with non-spastic CP, particularly hypotonic CP [32, 33]. In a population-based survey of more than 1000 children with CP from Iceland, Sweden, France, and the United Kingdom. The mean prevalence rates of co-occurring ASD were 8.7% (variation across centers ranged from 4.0 to 16.7%). This variation in prevalence rates most likely reflects the heterogeneity of study populations. It may suggest that ASD and CP share some underlying risk factors. Like many neurodevelopmental disorders, these rates of co-occurrence suggest that CP and ASD affect males more than females but appear more common in children with less severe functional motor limitations. Additional risk factors include epilepsy and intellectual disability [31, 34, 35].

Overall, the prevalence of behavioral and mental health disorders in children and adolescents with CP is higher than

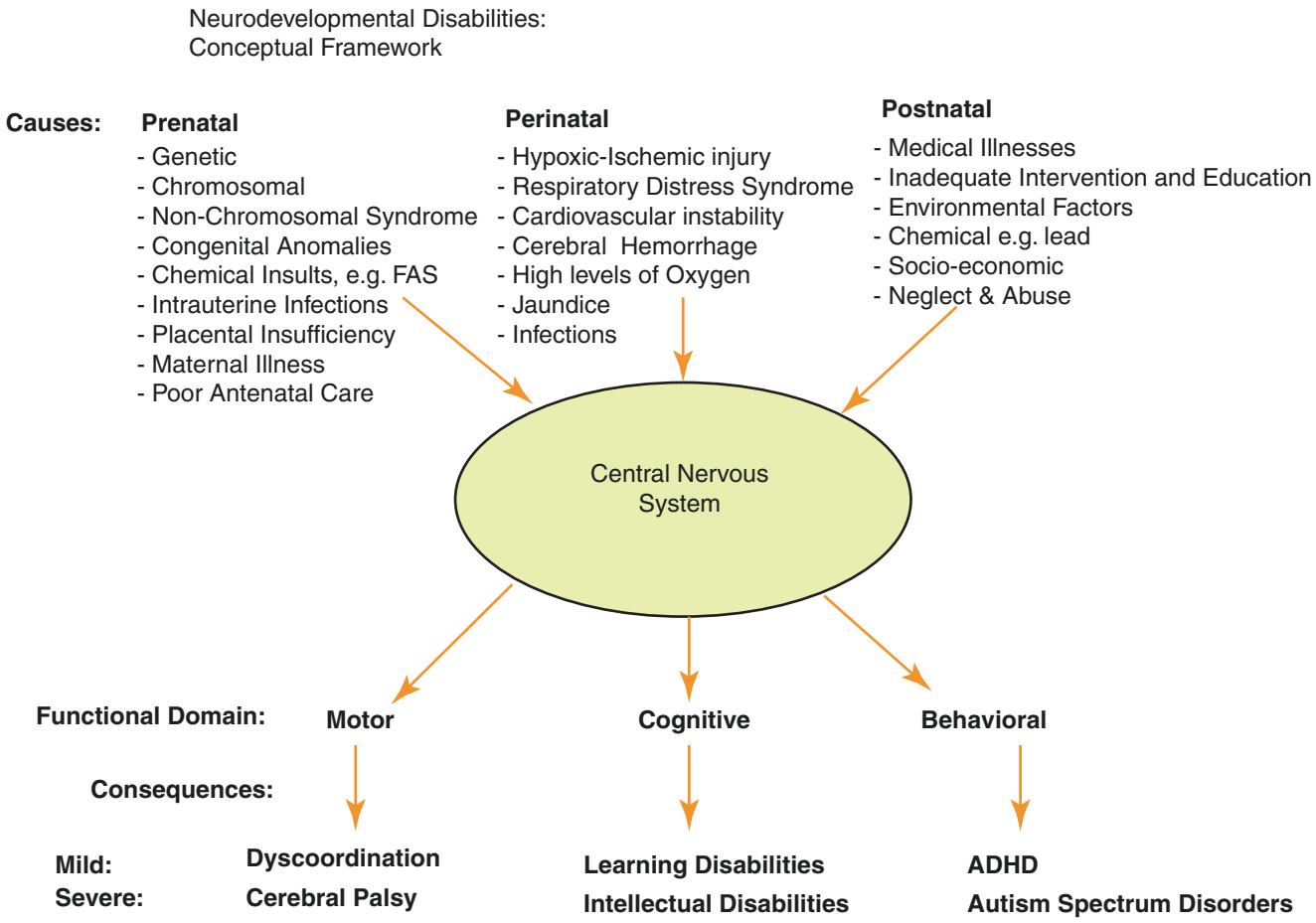

Fig. 40.3 Neurodevelopmental disabilities: a conceptual framework [9, 10]

the general population. In a meta-analysis of over 3000 studies support the need for standardization of assessment protocols. For example, the Child Behavior Checklist (CBCL) yielded a prevalence rate of 28%. In contrast, children given the Strengths and Difficulties Questionnaire had a prevalence rate of 35%. Psychiatric interviews revealed a prevalence rate of 57% [35]. This discrepancy may reflect difference referral biases but should remind clinicians that children with CP and intellectual disability are at greater risk of mental health disorder. Longitudinal studies suggest that the risk of anxiety and depressive disorders is not firmly associated with intellectual disabilities in children with CP [32].

The prevalence and nature of behavioral and mental health disorders among children with CP vary with three critical factors:

The etiology of the CP: As mentioned above, children with localized lesions are less likely to have associated neurodevelopmental disorders and they are similarly less likely to have behavioral and mental health disorders, although they are more likely than the general population to have emotional and social difficulties. One explanation hinges on physical appearance and functional difficulties. Etiology may also be a factor. A linkage between other neurodevelopmental disorders is present in children with congenital infections like CMV, known genetic or chromosome anomalies, hypoxic-ischemic subgroups, and acquired CP secondary to traumatic insults [36].

Severity of CP: The assessment of severity of CP as determined by the Gross Motor Functional Classification Scale (GMFCS) taps into the relationship between etiopathogenesis and severity of CP subtypes [37]. From this data, clinicians can conclude that extensive the CNS involvement correlates with neurodevelopmental, medical, and behavioral outcomes. There are realistic limits to the assessment of emotional and behavioral symptoms in children with severe functional limitations. The interpretation of emotional states and behaviors often depends on learning to interpret basic physiological or primary emotional responses [38].

Life experiences: Children with CP grow up with families that range in abilities to accentuate resilience and developmental health, or increase the risk for temperamental mismatches, attachment disorders, and behavioral-psychiatric disorders. Children with special health care needs

can overwhelm a family's ability to meet the demands on time, levels of physical and emotional resources, finances, and family adaptability and resilience. Many children can do well in stable families, but they are sensitive family dysfunction, psychopathology, substance abuse, availability of food, living arrangements and safety insecurities, and other adverse events. The child presenting with severe behavior challenges adds to these stressors and increases the risk for child maltreatment, including neglect and/or abuse [39, 40].

Bullet Points:

- Cerebral palsy is a complex developmental neurological disorder characterized by motor abnormalities.
- Several phenotypes exist but etiopathogenesis is tied to the nature and degree of brain development and maturation.
- Epilepsy, ASD, IDD, academic/learning disorders, and ADHD are frequently observed.
- There is an increased risk for significant disruptive behavioral and co-occurring psychiatric disorders.
- Children are vulnerable to a host of ACEs that play a major in most behavioral problems.

Conclusion

Children with CP are more likely to have associated neurodevelopmental disorders such as learning disabilities, intellectual disabilities, and medical complications such as seizure disorders. They are also more likely to have emotional, behavioral and mental health disorders (including ASD). The levels of family distress may overwhelm the parents and contribute to the risk for abuse and neglect. In light of these complications, it is imperative to examine and address the physical, medical, school performance, and academic progress, as well behavioral issues. The clinical team needs to focus on family dynamics and functional well-being and hopefully detect and intervene early whenever major problems arise [32, 39, 40].

Fetal Alcohol Spectrum Disorders FAS (D)

Claire D. Coles

Pathology and Pathophysiology

Alcohol is a potent teratogen and maternal alcohol use during gestation is associated with fetal alcohol spectrum disorders (FASD). Alcohol exposure during the first trimester contributes to the facial features [41] that characterize the disorder and during the third trimester, to growth deficiencies [42]. However, alcohol appears to have detrimental effects throughout gestation and abstinence is the most conservative approach for assuring positive birth outcomes. The United States Surgeon General has indicated that there is no "safe" level of alcohol use during pregnancy [43].

There does not seem to be a single mechanism for the teratogenic effects of alcohol. Researchers attribute the transcription effects of mRNAs across gestation and onto in postnatal development [44–46]. In addition, alcohol/polydrug use in pregnancy frequently co-occurs in the context of poor prenatal care, [47], nutritional deficiencies [45], and an increased risk for preterm birth [48].

Central nervous system (CNS) involvement includes microcephaly, seizures, motor pathology, and intellectual and behavioral deficits [48]. Neuroimaging studies of children with prenatal alcohol effects (subclinical subtype) display volume reduction in both grey and white matter, functional and structural connectivity deficits, and alterations in functional efficiency [49, 50].

The spectrum: The variability of prenatal alcohol effects of suggests that this exposure syndrome is really a spectrum of signs and symptoms—alcohol-related neurodevelopmental disorders (ARND). Recently, Mays et al. [51] estimated that up to 5% of American children lie on this spectrum. The DSM-5 uses Neurobehavioral Disorder-Prenatal Alcohol Exposure (ND-PAE) to capture this diversity [10].

Clinical Characteristics and Diagnosis

In 1973, Jones and Smith proposed four core criteria for the diagnosis of FAS. The Institute of Medicine refined these further [51]. These are as follows:

1. Evidence of prenatal exposure to alcohol at levels consistent with "risk"
2. Characteristic physical features, sometimes limited to several "sentinel" facial features, particularly small palpebral fissures, absent or indistinct philtrum and thinned upper vermillion
3. Growth retardation either at birth or subsequently at the third or tenth percentile depending on the system used; and
4. Neurobehavioral deficits associated with the impact of the teratogenic exposure on the central nervous system (CNS) that affect cognition, motor skills, and behavior.

Several diagnostic systems presently in use in North America rely on these criteria. Those most commonly used in North America are the Seattle 4-Digit Coding System [52]; the Hoyme et al. revised Institute of Medicine criteria [53], and the Revised Canadian System [54]. Many clinicians opt to use the IOM criteria in an informal manner as a guide to diagnosis.

Behavioral and Mental Health Concerns

Although FASD relies upon prenatal alcohol exposure (PAE) and characteristic dysmorphology, CNS affects are the most problematic. The CNS effects may have lifetime implications. FASD alters brain development and maturation. As infants, affected individuals show sleep disturbances, eating difficulties, and inability to regulate their state. As infants, affected individuals show sleep disturbances, eating difficulties, and problems with state regulation. These alterations occur in conjunction with late emerging deficits in cognition and behavior problems. PAE also adversely affects developing motor skills, language, cognition, behavior, and academics. Behavior and language disorders characterize the preschool period and persist into school age when learning problems often manifest as well. Cognitive problems can include intellectual deficits, borderline cognitive functioning as well as "average" ability with specific areas of deficit, like executive functioning skills, or math disability. In adolescence, impairments in executive functioning (i.e., judgement and planning) can interfere with academic and vocational achievement. There is some indication that depressive disorders may be more common in this group [55, 56]; and attention deficit, hyperactivity disorder (ADHD) is frequently diagnosed [57].

Social Context

Substance abuse is one of the most common reasons for loss of custody and many children whose mothers used alcohol in pregnancy have experienced inadequate caregiving. Individuals with FASD who present clinically are often not in the care of their birth families. In some cases, this has included significant neglect (including medical neglect) and abuse. In addition, women giving birth to children with full FAS often do not live to see the child grow up secondary to persistent alcohol use. Many children end up in foster care or adopted. Others remain in homes with one or two parents who continue their substance abuse. These households are rife with abuse/neglect, ongoing substance abuse, financial stress, marital instability, and a lack of access to or use of social resources. These children grow up in the face of overwhelming ACEs [7, 55].

Behavioral and Mental Health

Mental health issues are common in FASD. The pathophysiology of these disorders represents and amalgamation of primary and secondary conditions. The teratogenic effects of alcohol derail the hierarchical organization of brain pathways that underlie cognitive, academic, and behavioral/mental health disorders. A number of ACEs add to these uncertainties but do not condemn the child to mental illness. The sources of their resilience are still under investigation. Affected children, however, frequently present with externalizing disorders, demonstrating significant problem with arousal and affect/behavioral regulation [54–57].

In the first years of life, this may manifest as sleep disturbances (frequent waking, inadequate sleep), frequent tantrums, and difficulty in achieving age-appropriate levels of self-regulation. By the preschool period, the child may appear to have problems with "attention" and oppositional behavior. Developmental delays in speech/language and cognition exacerbate problems mastering socialization and adjustment. School-aged children are vulnerable to significant distress secondary to social adjustment problems and/or academic difficulties. Although many present with ADHD-like symptoms, they may also show depression and anxiety. In adolescence, young people may have difficulties in meeting socially dictated standards of independence or achievement resulting in maladjustment, acting out behaviors, and mental health problems. Young people who have issues with executive functioning, which is common in FASD due to the impact of alcohol on the prefrontal cortex, may exhibit significant problems in judgement leading to risky behavior, including substance abuse, sexual acting out, or legal problems [58, 59].

The question of ADHD. Frequent misdiagnose of primary ADHD occurs in children with FASD in spite of significant clinical differences in clinical presentations [57]. Most noteworthy is the limited efficacy of psychostimulants. This observation suggests a different behavioral neuropharmacology and functional brain activity. Individuals with FASD have difficulties in encoding new information and, significantly, in "switching" attention when this is a more effective strategy. They do not appear to have significant difficulties with focusing or sustaining attention, which is common in ADHD [58, 59]. Both may exhibit externalizing behaviors or be noncompliant.

Executive Functioning and FASD. Based on both neuroimaging and behavioral studies, the role of deficits in executive functioning is central to FASD. Executive functions include memory, aspects of cognitive control (working memory, attention), and metacognition (thinking about thinking). These deficits adversely affect social judgement, planning, organization, and understanding the long-term effects of their actions [59].

Treatment of FASD. There are few studies of interventions specific to FASD, although these do suggest that appropriate interventions are effective [60]. Medication trials are generally unsuccessful for many of the behaviors associated with FASD [61]. It is also necessary to view the child in the social context to assure that the most effective approach is used.

Educational Concerns

Neurocognition is a central issue in FASD. Approximately half of those diagnosed with full FAS have IQs less than 70, placing them in the intellectually disabled range. Others are functioning the "Borderline range" with IQ from 70 to 85, which places them at a significant disadvantage in a classroom with typical children. Finally, even among those with higher IQs, many have specific learning disabilities that make academics more difficult. Less cognitively impaired children may still present with specific learning and motor skill learning difficulties. One frequently observed pattern is the discrepancy between relatively "average" verbal skills and significantly impaired nonverbal and spatial skills. This is a kind of "hidden" learning disability often associated with math disabilities and problems in social functioning (nonverbal learning disability). These deficits require psychoeducational and neuropsychological evaluation to assess potential academic achievement and ability to learn and function in school settings. Determining these needs can permit the prescription of matched habilitative interventions specifically tailored to meet learning and academic needs [62–64].

Transition to Adulthood

Like most individuals with special needs, young people with FASD need support to achieve an optimal transition to adulthood [65]. Even under the best of circumstances, the transition to adulthood is a very challenging time. Individuals with developmental disabilities shift from school and other programs to adult social roles and occupational demands. Many young adults with FASD require extensive vocational resources, as well as supportive living-group homes with support resources. Those with "average" cognitive abilities may not be able to access these necessary services due behavioral, educational and legal shortcomings.

It is important for families to begin transitional planning early and to use services available through schools and governmental agencies to ease the transition as much as possible. Seeking support from professionals can be helpful at this time. Therapists can help in making realistic plans for the future. It can be difficult to accept that a child will not attend college, for instance, but understanding this can be a relief to the affected adolescent who can then begin to explore other options. There are also lawyers specializing in family law who can guide parents through planning for guardianships and trusts. Although there has not been extensive information about this transition for FASD specifically, information is available about other disabilities that can be very helpful [62–65].

Attention Deficit Hyperactivity Disorder (ADHD)

Jarrett Barnhill

Introduction

ADHD is a complex neurodevelopmental disorder affecting the regulation of motor activity, impulse control, attention, and vulnerability to distraction by both internal and external, competing stimuli. These core deficits belong to a larger class of executive functions devoted to the top-down regulation of subcortical, limbic, motor, and reward pathways [13, 66, 67].

ADHD also frequently co-occurs with other neurodevelopmental disorders (NDs). These include ASD, IDD, traumatic brain injury, CP, epilepsy, FASD, and other toxin exposures, adverse life experiences, and as a major player in behavioral and psychiatric comorbidities [68]. The cooccurrence of ADHD in disorder such as ASD and IDD frequently alters their clinical presentation and create additional management issues. The burden of deficits in Executive Functions associated with ADHD adds to pre-existing deficits in higher order cognitive, memory, motor functions, developing motor and social skills, emotion regulation and externalizing disruptive behaviors, aggression, and self-injury [20, 59, 68–70].

Psychostimulants remain the mainstay of management for uncomplicated ADHD, but non-stimulant alternatives are widely prescribed in patients who cannot tolerate stimulants or with psychiatric comorbidities [69]. Combined psychopharmacological (stimulants plus other psychotropes), or the augmentation of pharmacotherapy with behavioral, psychoeducational, and cognitive psychotherapies are for children with significant social skill deficits, specific learning disabilities, psychiatric comorbidities, trauma and other aversive life events, and dysfunctional environmental support systems. Combined therapies are also beneficial in relapse protection and as a support during attempts to wean the child from medical treatments [69–71].

The Evolution of Attention Deficit/Hyperactivity Disorder

Historically, ADHD is a bit of a shape shifter. Originally, clinicians focused on hyperactivity/hyperkinesis as the defining characteristic of ADHD. The introduction of dextroamphetamine more than 80 years ago proved helpful for not only hyperactivity but also affect regulation, disruptive and aggressive behavior, hyperactivity, and impulsivity. The target population in the late 1930s were children with co-occurring developmental insults such as CP. The next step in ADHD narrative shifted to include children with cognitive impulsivity, inattention, motor and learning disabilities. The concept of MBD (initially as minimal brain disorder, then changed to

minimal brain dysfunction) encompassed these symptoms as well as emotional dysregulation/irritability, subtle neurological soft signs, and dysmorphic changes that suggested of early gestational challenges. Psychostimulants became the treatment of choice, but the list of treatment alternatives expanded quickly for nonresponders [13, 66–70].

It was also apparent that there was a gender dimorphism among children with ADHD and many other NDs. For ADHD the male to female ratio was 4:1 in most population studies. The male gender bias arose in part because of the focus on the overshadowing by less disruptive attentional deficits. More recent studies suggest that many females fell through the cracks secondary attention deficits, patterns of comorbidity (internalizing disorders), and white matter developmental trajectories [13, 70, 71]. These data suggest the under diagnosis of females and treatment focused on other psychiatric comorbidities [13].

The DSM 3–5 elevated inattention/distractibility to hyperactivity/impulsivity and broke up the remnants of the original MBD into specific disorders. During this era, the diagnosis of ADHD focused on it as a disorder of attention with a dash of other dysfunctional executive skills such as emotional dysregulation and reward processing deficits [71, 72]. There was also a growing recognition that ADHD did not fade during adolescence but in fact persisted into adulthood.

Acceptance of the of adult ADHD lagged since many patients no longer met the diagnostic criteria (mainly gross motor hyperactivity and impulsivity) or developed substance abuse or other psychiatric disorders. Additional research challenged this clinical pessimism. Adults struggled with persisting neuropsychological deficits in spite of attenuated gross motor hyperactivity/impulsivity and negative effects on social-emotional relationships and occupation performance. Factors associated with persistence included genetic vulnerability and increased demands for attention and other complex executive functions. The continued use of stimulants for adults met additional barriers secondary concerns about substance use disorders, externalizing personality disorders, and the co-occurrence of attentional and impulse issues in several major psychiatric disorders [70–75].

In recent years, focused neuropsychological, neurophysiological, and clinical studies suggest that attention is more complex than originally understood. Neuroscientists and other researcher raised questions about the nature of inattention and began shifting focus towards a new concept Intention Deficit Disorder. This shift reflects the developing capacity to select relevant stimuli; sensory gating of competing stimuli; intentionally sustaining task related performance; responding to stop/start signals, monitoring performance to task, and disengaging or shifting sets when completed. These executive functions require engaging multiple cortical/subcortical networks to task demands and a great deal of top-down regulation [68, 71, 76, 77].

Pharmacogenomic studies provide evidence that although inattentive and hyperactive/impulse specifiers comprise ADHD, the genetic relatedness of these domains remains relatively weak. Nearly 75–80% of children with uncomplicated hyperactive/impulsive ADHD (ADHD simplex) respond to stimulants. The response rates of the inattentive subgroup are more variable, further suggesting the two subgroups differ along several neurobiological and neuropharmacological domains [72, 77].

The goal of therapy is to attenuate these behavioral excesses (impaired top-down regulation) sufficiently to allow the developing child to master skill deficits and learn new patterns of cognition and behavior. In short, pharmacotherapies assist in developing self-regulation skills but they do not teach new social or academic skills [68, 70].

Overview of Neurodevelopmental Disorders

The DSM-5 introduced the section on neurodevelopmental disorders (ND) addresses a subset of childhood onset syndromes with lifelong implications. Since ADHD and ASD are included, it is prudent to discuss this group in more detail (Box 40.1). This outline expands the core features of NDs in order to demonstrate not only the interrelatedness between the syndromes listed below but also to set the stage for the emerging concept that most primary psychiatric disorders are neurodevelopmental in nature [20, 23, 24, 71, 78].

1. The diagnostic criteria for many neurodevelopmental disorders require the presence of deficits across multiple domains; age of onset/recognition during the developmental period, performance discrepancy criteria on standardized intelligence or psychoeducational testing as compared to neurotypical populations.
2. Exclusion criteria for this group include ruling out genetic/metabolic, neurological, and other neurocognitive disorders. Comprehensive assessments require medical and

Box 40.1. List of Neurodevelopmental Disorders

Intellectual disability (intellectual developmental disorder
Communication disorders
Autism Spectrum disorder
Specific learning disorder
Motor disorders- includes tic disorders, CP
Other specified developmental disorders- FASD and CP
Unspecified developmental disorder [13, 66]

genetic histories; detailed physical examination and laboratory testing; observational data from multiple sources, and the use of normalized rating instruments [13, 66].

3. Age of onset: Criteria focus the emergence either a specific age or "during the developmental period." Although this criterion seems straight forward, brain development and maturation are ongoing processes that continue well into young adulthood. In many cases, individuals with severe ID (IDD) and ASD the age of recognition may be more useful than age of onset [13, 67, 68].
4. Complex etiopathogenesis: The eventual phenotypes for most neurodevelopmental disorders arise from a complex amalgamation of gene X environment/ecological interactions. These multi-directional interactions influence ongoing neurogenesis, neuronal migration and maturation; myelination, and the development of neuronal networks devoted to higher both regulatory and higher cortical functions [71–73].
5. Impact on later brain development: Neurodevelopmental disorders include a range of severity, pervasiveness of functional impairments and a hierarchy of cognitive disabilities and social functioning. These include diminished cognitive/behavioral flexibility, global deficits that result in impaired skill learning across conceptual, social and practical living skills, and academic/occupational performance domains [13, 74, 79].
6. Variability in gender distribution. Aside from ID, NDs display higher prevalence rates for males suggesting a degree of gender dimorphism in the complex interactions that influence phenotypic expression, as well as patterns of learning and social development. Some of these differences may be related to selection biases that contribute to the under diagnosis of females. A similar finding may also apply to the early recognition of ASD [68, 70, 74].
7. Neuroimaging: Additional neurobiological studies suggest that we study ADHD in terms of the relationship between gene markers (and gene x environmental interactions) and functional neuroimaging. Combing these studies with neuropsychological testing provides useful insights into the timing and development of multiple interconnected, neuronal networks pathways. These translational studies are also providing a deeper understanding of the development of higher order memory functions, motor planning, goal selection, set shifting, and other top-down aspects of attentional cognitive, emotional, and motor regulation (executive functions) [74, 80]. Disruptions in circuitries can affect late developing sensorimotor, cognitive, academic, and social and communication skills that contribute to the emergence of behavioral and mental health disorders [71, 72].
8. Genetics: Genome wide /exosome analyses suggest a degree of phenotypic and genomic overlap between many NDs ADHD, autism spectrum disorder (ASD), intellectual disability (intellectual developmental disorder, ID (IDD), other specific learning disorders, schizophrenia, mood disorders and epilepsy [11, 72]. Epidemiologically, the high rates of co-occurrence between ADHD and ASD, IDD and ADHD, motor skill development and specific learning disabilities, as well as ADHD, epilepsy, and tic disorders, suggests a group of complex polygenic disorders [72, 76].
9. In addition to genomic influences, these studies help us understand the many transactional relationships between these NDs (including ADHD) and social, economic, nutritional, familial, psychological influences. For example, repetitive trauma and chronic stress may derail the development of prefrontal and association functional connections [24, 80, 81].
10. Variability and resilience: Factors associated with resilience and access to positive learning environments may attenuate the adverse effects [70]. The goodness of fit between a child with vulnerabilities, security of attachment, temperamental traits and their social ecology, and environmental stressors are also key factors in the development of internalizing, externalizing, and disruptive subtypes of primary psychiatric disorders [71, 79].

This abbreviated list suggests that ADHD, ASD, CP, and FASD qualify as neurodevelopmental disorders. Each represents a form of atypical brain development and maturation associated with gene dysregulation and changes in the multiple neuronal signals that drive the sequential/hierarchical organization of major neuronal circuitries and networks. They differ in terms of the timing and extent of these disruptive events. These core elements underlie temperament, attachment, and responses to environmental and ecological challenges that underlie behavioral and mental health disorders.

Diagnostic Criteria for ADHD

This section will focus primarily on the diagnosis of the five subtypes of ADHD. Each represent a configuration of two basic components-predominance of attentional deficits, or hyperactivity/impulsivity, or a combination of both. In addition, two subsyndromal subtypes fail to meet the categorical requirements, but may still contribute to significant functional impairment, and increased risk for psychiatric comorbidity [13, 66, 67].

ADHD diagnostic criteria

In both nomenclatures, attention deficit hyperactivity disorder represents a complex syndrome characterized by the following features (Box 40.2):

Box 40.2. Diagnostic Criteria for ADHD

1. Persistent pattern of inattention and/or hyperactivity-impulsivity that present with demonstrable functional impairments in the developmental trajectory of social, behavioral, executive functions, cognitive and emerging academic skills.
2. The upper limit for age of onset is 12 years old; duration of symptoms is at least 6 months—Persistent and pervasive symptoms noted in multiple settings (e.g., home, school, and work settings)
3. Symptom clusters- predominantly inattention (presence of 6 or more criteria, lasting 6 months, and recognizable level of functional impairment: Lack of detail focus, careless mistakes, sustained attention, distractibility in social settings, not following direction, finishing tasks, organizing and carrying, completing tasks, avoidance, losing possessions, forgetful of daily activities.
4. Hyperactive-impulsive- similar duration and qualification criteria: Restless/fidgety, frequently out of seat, inappropriate levels of motor activity, play quietly when called for socially, on the go, excessive talking, impulsive responses, difficulty waiting or taking turns, interruption and intruding in conversations.
5. Specifiers: Include subtypes (combined, predominantly inattentive, hyperactive/impulsive, severity, level of remission, other unspecified, unspecified

ADHD is a heterogeneous disorder that needs differentiating from multiple neuropsychiatric disorders. The differential diagnosis becomes the basis for exclusion criteria (rule-outs). One source of this diversity is the polygenic nature of ADHD and the multitude of gene markers (alleles). Most have small effect sizes, occasional larger copy number variants (CNVs), and pleiotropic genes that add to the confusion [72]. In addition, Genome Wide Association Scans yield shared gene markers for schizophrenia, mania, mood disorders, specific learning disabilities, epilepsy, ID (IDD), and ASD [71, 72, 81, 82].

Co-occurring and Comorbid Conditions

In spite of high heritability risks (60–80%) for ADHD, there is still some uncertainty about which genes are responsible. Like most polygenic disorders, there are multiple gene markers but each may have a small effect size and their influence diluted. In addition, epigenetic and pleiotropic effects cloud the picture. In addition, many of these gene markers are also present in the genomic scans of other neuropsychiatric disorders [72].

The ADHD phenotype is also a product of multiple toxicological and ecological factors sources of variability. The most commonly cited are intra-uterine/prenatal complications factors, pregnancy and birth complications, psychosocial adversity, and exposure to nicotine, alcohol and other environmental toxins [67, 70, 71]. The resulting gene X environmental interactions may partly the male predominance for many neurodevelopmental disorders. It may also underlie the apparent greater gene loading for female expression of other neurodevelopmental disorders. On the other hand, researchers probably missed female children in part because of preponderance of attentional rather than hyperactive/impulsive symptoms. Psychiatric comorbidity may also differ in terms of predominant internalizing behavioral and mental health disorders [74, 80].

ADHD is also frequently associated with ASD and ID (IDD). Paradoxically this recognition of this relationship floundered in part because until the publication of the DSM-5, ASD and ADHD were mutually exclusive diagnoses. Yet, clinicians still struggle with the boundaries between attentional deficits, atypical social behaviors and social communication issues that separate ADHD from ASD [66, 67, 73, 78, 82].

The relationship between ADHD and IDD is more complicated. The symptoms of ADHD appear linked to both chronological and developmental age, but this linkage is not clear-cut for children with severe-profound IDD. In addition, children with ADHD+IDD also share traits with those presenting with ADHD + ASD, ADHD + CP and ADHD +FASD and other neuropsychiatric disorders [18, 24, 32, 58]. In other words, can the ADHD criteria be adapted to developmental age and level of impairment based on deficits in conceptual, social and practical domains associated with IDD. This issue is especially germane for patients with severe/profound ID (IDD) in which epilepsy and other conditions, severe disruptive, self-injurious or aggressive behaviors overshadow the diagnostic criteria for ADHD [13, 67].

These complications also apply to the risk for psychiatric disorders. Deficits in adaptive and cognitive abilities increase a child's vulnerability to both trauma and stress related, but also influence genetic and psychosocial triggers for other psychiatric disorders. In many situations, aggression, self-injury and disruptive behaviors and withdrawal overshadow symptoms of both ADHD and other psychiatric disorders. Under these circumstances, diagnosis becomes as much hypothesis testing as reaching a definitive diagnosis and constructing a treatment plan to match that diagnosis. For example, clinicians may assume that children with ADHD + ASD or ID (IDD) should avoid stimulants due to the presence of severe disruptive behaviors. Other studies

note diminished response to psychostimulants, and a greater risk for adverse effects than neurotypical children [10, 72]. As a result, treatment focuses on antipsychotics, mood stabilizers, SSRIs or polypharmacy and stimulants are frequently overlooked [67, 70, 83].

Assessment

A comprehensive assessment requires a team of experienced clinicians who can investigate not only genetic, medical, neurological, psychological/psychiatric and psycho-educational histories, but also provide comprehensive physical and neurological examinations and diagnostic testing but also seek information regarding family, psychosocial and interpersonal profiles. The goal is to understand the ecological context of the child with ADHD. The psychosocial setting shapes social and cognitive development, academic achievement, and psychosocial adjustment and contributes to either resilience from or vulnerability to behavioral and mental health disorders. Each of the factors contributes to success or failure of the treatment plan [66, 67, 70].

Trends in Treatment

The psychostimulants emerged in the mid 1930s psychostimulants as the mainstay for treating ADHD. In the ensuing 80 rears, we witnessed the release of multiple new formulations of this class of psychotropic drugs emerged. In addition, thousands of scientific articles addressed their use, molecular pharmacology, pharmacokinetic properties and randomized double blind controlled studies assessing their efficacy. Since the early 1990s, the MTA (Multiple treatment of ADHD), and follow-up meta-analyses of this landmark study suggested that 70–85% of children with uncomplicated ADHD respond to psychostimulant monotherapy. The selection of behavioral and other manualized psychotherapies, or combined pharmaco/psychotherapies may still benefit subsets of children who have incomplete responses, comorbid NDs (including FASD, CP), and co-occurring primary psychiatric disorders [70, 84, 85].

When confronted with co-occurring or comorbid mental health disorders, most drug treatments lead to improvement, not complete remission or "cure." In addition, there are nonresponders who remain symptomatic or get worse with treatment. Listed are several steps that may help finding a potential solution to the conundrum—the four wrongs.

1. Wrong child (misdiagnosis or overlooked genetic, medical, prenatal alcohol/drug exposure, and a plethora of neurological and metabolic conditions)
2. Wrong family (high levels of family dysfunction, substance abuse, trauma and parental psychopathology);
3. Wrong information such as incomplete data tracking, miscommunications, and incomplete compliance or overt noncompliance.
4. Wrong drug, dose, recognition of drug-drug interactions (pharmacogenetic effects) or adverse drug side effects (modest response to stimulants, or lower threshold for adverse effects from stimulants in children with ASD) [70, 84].

Stimulant nonresponse should remind the clinician of the considerable heterogeneity among children with ADHD [71, 72]. In addition, the growing application of increasingly sophisticated functional neuro-imaging, pharmacogenomic, neurophysiological, and neuropharmacological data reinforces the awareness that ADHD defined descriptively (DSM-5 criteria) is a complex disorder with multiple phenotypes and predisposing factors that follow different developmental trajectories. Intertwined with these developmental mosaics are gene polymorphisms that influence pharmacokinetics and pharmacodynamics of stimulants and other effective psychotropes [72, 84]. These situations also remind clinicians and neuroscience researchers that that we probably learn more from atypical cases grouped among the treatment nonresponders than we do from responders.

Summary

ADHD is a complex neurodevelopmental disorder that challenges many of our older concepts about genetics, the transactional nature of brain development maturation, the neuropharmacology, neuropsychology, and functional neuroimaging of attention and top-down regulation. Perhaps the biggest challenge was to our belief in the selective nature of categorical, diagnostic nomenclatures.

Our comfort level with gender ratios of both ADHD and ASD is more complex than we understood for other neurodevelopmental disorders. The overlap between ADHD spectrum and other psychiatric disorders suggests the gender differences in comorbidities may be quantitative (dimensional) and qualitative (categorical). For example, males are more commonly diagnosed with the hyperactivity/impulsivity or combined subtypes of ADHD as well as motor, reading and other specific learning disorder, as well as ASD, tic disorders, and externalizing disorders such oppositional/conduct/disruptive psychiatric disorders [70, 85–88].

Females display a slightly different pattern of risk. There is a tendency, for females, to present with the Inattentive subtype and other internalizing symptoms that overshadow the recognition of ADHD [13, 67, 80]. These trends may also relate to the distribution of many internalizing versus externalizing dis-

orders. These data differ also suggest a bias in vulnerability to "secondary" forms of ADHD for males. Data from studies of persistence of ADHD suggest stronger genetic ties to parents rather siblings, probably related to polygenic inheritance. In the past, several explanations for gender differences included greater genetic for symptomatic females than males; and a greater for or vulnerability to prenatal, pregnancy, and delivery complications; psychosocial adversity and exposure to toxins may account in part for the differences [72, 87, 89].

The majority of adults express reduced gross motor hyperactivity/impulsivity, but may retain inattentiveness. Many continue to express vestiges of impulsivity in emotional, cognitive, academic, and social domains. The male: female ratios may shift except in circumstances where comorbid mood and anxiety are present. Response to psychostimulants remains, but many adult patents end up on combined treatment regimens secondary to comorbidity, or substance abuse in many clinical settings. Although we did not emphasize various modes of psychotherapy, these interventions remain a necessary element for many patients with ADHD. This is especially true for those with psychosocial adversity, marital dysfunction, psychiatric comorbidity, and substance use disorders [87, 89].

The developmental trajectory and treatment course for ADHD are more of a shrubbery with intertwining limbs than a linear process. Part of the complexity resides the heterogeneity of ADHD as a mixture of syndrome, trait functions, persistent symptomatology into adulthood (homotypy), and a group of children with ADHD-like symptoms that appear to be a nonspecific presentation of later-onset psychiatric comorbidities (heterotypy) [90, 91]. For example, children with ADHD are at risk for disruptive mood dysregulation disorder (DMDD), major depressive, anxiety [18], bipolar CD/antisocial personality and substance use disorders that may overshadow their childhood ADHD [79, 87, 89].

Summary Bullets

- ADHD is a highly heritable neurodevelopmental disorder characterized by dysregulation and impairments in attention and hyperactivity/impulsivity.
- In well-diagnosed children, 80+% are likely to respond to one of the many stimulants on the market.
- ADHD is a heterogeneous syndrome that most likely represents a final common pathway from multiple neurobiological/environmental sources.
- ADHD is frequently comorbid with multiple cognitive, neurological, behavioral, and primary psychiatric disorders.
- Stimulant nonresponders are generally intolerant of stimulants, experience adverse side effects, or have significant cognitive, developmental, and psychiatric comorbidities.

Chapter Discussion

In this chapter, the authors provided an overview of ASD, CP, FASD, and ADHD. It is clear that ADHD and ASD are complex, polygenic disorders. Many of the genes they share multiple gene markers also differ in their impact on hierarchical organization of neuronal circuitries and developmental trajectories [26, 76, 77, 79, 91] These differences relate to the expression of diverging patterns of atypical neurogenesis, neuronal migration, maturation, synaptogenesis, myelination, and neuronal network development and function. Similar patterns of gene dysregulation and atypical neuronal development occur secondary to prenatal exposure to infectious, environmental toxins, and maternal inflammatory and autoimmune disorders [26, 76, 77, 79].

During later phases of brain maturation, severe childhood stressors and trauma disrupt the entrainment of the stress-response system (hypothalamic pituitary axis and the balances between sympathetic/parasympathetic activation). Both gene dysregulation and epigenetic changes disrupt emerging executive functions, cognitive, memory, motor and social skills. For example, brain insults (CP) and exposure to substances of abuse disrupt developing neuronal circuitries that underlie patterns of temperament, attachment behaviors, top-down regulation of emotional and behavioral responses [25, 62, 70, 72].

The differences between CP, FASD, ADHD, and some secondary forms of IDD and ASD are in part due to the timing and extent of atypical brain development. As note earlier, children with ASD differ from those with ASD + ADHD in terms of the severity of deficits in executive functioning and the distribution of externalizing/disruptive behaviors associated with high functioning ASD. The phenotype of ASD + ADHD+ FASD is less well defined but clinically relevant to the level of service needs. Children with ASD+ ADHD+CP phenotypes may struggle with complex epilepsies, speech and language disorders, and disruptive behaviors. This combination may increase the likelihood of both social communication deficits and repetitive and restrictive behaviors as well as self-injury, destructive aggressive, and other externalizing/disruptive behaviors. Children with severe ASD, ADHD+ severe ASD+ severe IDD+/− CP, and /or FASD can overwhelm the carrying capacity of many community services [14, 19, 36, 74].

Each of these groups are at increased risk for behavioral and mental health disorders. The problems for many clinicians, however, are grappling the complexity of presenting complaints, and organizing and developing effective treatment programs for these problems [88]. In many clinical settings, service providers and families facing critical shortages in treatment resources are looking to psychopharmacologists for solutions. We still lack definitive, disease-altering pharmacotherapies for most behavioral and psychiatric disorder in spite of major advances in the clinical neurosciences, genetics, and neuropharmacology [88–91]. Our work is not finished.

Multiple Choice Questions

1. In behavioral and mental health settings, wellness represents which of the following?
   (a) No overt signs of depression or anxiety.
   (b) Improvement and free of medication side effects.
   (c) A sense of emotional, psychological and social well-being.
   (d) Disappearance of Hallucinations and delusions.

Answer: (c)

Mental health is more than an improvement or absence of symptoms. Enhancing mental health involves resilience, relating to others, and making good choices. It is a preventative strategy that encourages problem solving and attenuates the risk for many mental health disorders.

2. Adverse Childhood Experiences contribute to which of the following?
   (a) Catching the flu before the prom.
   (b) Worrying about a basketball tournament.
   (c) A sense of safety and security.
   (d) Contributes to social, emotional and Cognitive impairment.

Answer: (d)

ACE's are negative life experiences that take a toll on social motional, and cognitive development. There effects can be cumulative and lifelong. High levels of ACE's increase the risk for poor academic performance, behavioral and mental disorders, substance use, chronic disease and can shorten the lifespan.

3. The diagnosis of Autism Spectrum Disorder requires the following traits:
   (a) The presence of auditory and visual hallucinations.
   (b) Age of onset age of onset in late adolescence.
   (c) Rarely associated with Intellectual Disability or other Neurodevelopmental Disorders.
   (d) Presence of social communication deficits and repetitive/restrictive behaviors.

Answer: (d)

Autism emerges early in child development, often before the age of two. IDD frequently co-occurs with ASD. The presence of auditory and visual hallucinations suggests the onset of other medical/neurological or psychiatric disorders.

4. The male: female gender ratio for ASD is:
   (a) There is an equal ratio of males to females
   (b) Females outnumber males in most families
   (c) Male are 4–8 times more likely to have ASD
   (d) Females are rarely diagnosed with ASD

Answer: (c)

Although the male female ratio ranges from 4 to 8, many females are likely to be overlooked due to between social skills and severity of restrictive/repetitive behaviors.

5. Meltdowns or severe tantrums are most likely to occur in children with ASD under the following conditions?
   (a) In situations that result in unexpected changes in routine or preferred activities
   (b) Upon awakening in the AM
   (c) When presented a favorite food
   (d) Among familiar peers engaged in preferred activities

Answer: (a)

Some children with ASD do have disrupted sleep but tantrums are not commonplace. Usually predictable situations, with preferred activities and preferred children are less like to trigger outbursts. The transition from such activities is another story.

6. Cerebral Palsy is a disorder of movement and posture characterized by which of the following findings?
   (a) Progressive neurological changes during development
   (b) Minimal overlap with other Neurodevelopmental Disorders.
   (c) IDD occurs in 40% of affected children.
   (d) Epilepsy is rarely associated with CP.

Answer: (c)

CP is included among the static encephalopathies without progression or degenerative changes. CP is frequently associated with a wide range of neurodevelopmental disorders and shows, including learning disabilities, ADHD, and IDD. Epilepsy occurs in 35% of affected children.

7. CP co-occurs in ASD in children with which of the following children.
   (a) Those in the superior ranges of measured intelligence.
   (b) Higher frequency in males versus females
   (c) Those with more severe motor symptoms
   (d) Those children without epilepsy.

Answer: (b)

ASD in children with CP is commonly associated with co-occurring IDD, epilepsy, and mild motor symptoms. The combination is more common in males than females.

8. In Fetal Alcohol Spectrum Disorder, facial dysmorphologies are more likely to occur in children with which of the following:
   (a) Significant alcohol exposure during the first trimester
   (b) Significant alcohol exposure during the third trimester
   (c) Rarely seen in children with FASD.
   (d) Growth retardation is usually not associated with FASD

Answer: (a)

FASD is a spectrum disorder with a wide range of clinical presentations. Continuous alcohol ingestion during the first trimester are linked to facial dysmorphology. Growth retardation can arise from alcohol exposure during many points.

9. Behavioral and mental health issues are more likely to occur in which of the following?
   (a) Across the entire spectrum of FASD
   (b) Rarely seen in children with significant neurocognitive deficits
   (c) Is unrelated to adverse childhood events
   (d) Has little impact on academic performance

Answer: (a)

The milder end of the FASD spectrum is also a significant risk factor for behavioral and mental health disorders. These children are vulnerable to cognitive and academic difficulties and can be especially vulnerable adverse childhood events.

10. In the DSM-5, Attention Deficit Hyperactivity Disorder belong to which group of primary psychiatric disorders?
   (a) Trauma and Stressor-related Disorders
   (b) Impulse control disorders
   (c) Neurodevelopmental Disorders
   (d) Anxiety Disorders

Answer: (c)

ADHD is listed among the Neurodevelopmental Disorders based on an early onset (prior to age 12). Among the Neurodevelopmental Disorders there is a high level of comorbidity and co-occurrence. Impulse control, trauma-related and anxiety are possible outcomes for ADHD but not directly related to othe Neurodevelopmental Disorders.

11. Which of the following are among the core symptom groups required to make the diagnosis of ADHD?
   (a) Age of onset before 12, inattention, hyperactivity/impulsivity across multiple settings
   (b) Hyperactivity/impulsivity, motor disorder, disruptive and destructive behavior
   (c) Inattention, externalizing, oppositional defiant behaviors, overt defiance
   (d) Onset in childhood that completely resolves during adolescence

Answer: (a)

Motor co-ordination, motor tics and affect dysregulation frequently co-occur in children with ADHD but are not diagnostic criteria. A similar line of reasoning applies to question c. Once it was common clinical lore that ADHD waned in late adolescence. This proved wrong and the 60% of adults with childhood ADHD have full syndrome or residual symptoms.

12. Which of the following characterizes the efficacy of psychostimulants in the treatment of children with ADHD?
   (a) The majority of children do not respond
   (b) Children and ADHD predominantly inattentive type are most likely to respond
   (c) Nearly 90% of children with uncomplicated ADHD respond to psychostimulants
   (d) Children with comorbid ASD or IDD should not be prescribed stimulants.

Answer: (c)

Nearly 90% of children respond to one of the psychostimulants. Children with ASD and IDD have lower rates of response and higher risk for side effects but many do improve. Children with ADHD hyperactive impulsive or mixed type are the best responders. Inattentive children may improve significantly but less dramatically than the hyperactive/impulsive and combined cohorts.

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Role of Gender and Neurodevelopmental Disabilities

41

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Learning Objectives

- Demonstrate a link between gender and some neurodevelopmental disabilities.
- Establish the potential role of the utero-placental unit in protecting the embryo-fetus from specific maternal stresses.
- Illustrate the influence of specific biochemical factors in male versus female vulnerabilities.
- Recognize research on the epigenetic role of O-linked N-acetyl-glucosamine transferase (OGT) in vulnerabilities of the male versus female gender.

Highlights

- Research continues to evaluate why some neurodevelopmental disabilities are more common in one gender versus another.
- Males in childhood have increased risks for attention-deficit/hyperactivity disorder, autism spectrum disorder, mental sub-normality, dyslexia, stuttering, and early-onset schizophrenia.
- During puberty, females have increased risks for eating disorder, depression and anxiety.
- Research notes that the utero-placental unit can help protect the embryo-fetus from material threats that include infection, drug use and trauma.
- Research over the past decades has led to the Barker hypothesis or fetal origins hypothesis in which conditions in pregnancy and during birth influence later health in animals and human beings.
- A key biochemical factor under study for links to gender vulnerabilities is O-linked N-acetyl-glucosamine transferase (OGT).

Introduction

The link between gender and neurodevelopmental disabilities has been identified for eons and remains under study in the early part of the twenty-first century. Table 41.1 provides the outline of this discussion. Males in childhood, for example, have a higher risk for attention-deficit/hyperactivity disorder, autism spectrum disorder, mental sub-normality, dyslexia, stuttering, and early-onset schizophrenia (EOS), while pubertal girls are at increased risk of other conditions—such as eating disorders, depression, and anxiety [1–3]. This discussion considers the relationship between gender and neurodevelopmental disabilities while also reflecting on potential etiologic underpinnings in this link that includes in utero epigenetic factors involving O-linked *N*-acetylglucosamine transferase (OGT) [4–8].

Table 41.1 Outline of role of gender and developmental disabilities (DD)

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Neurodevelopmental Disabilities and Males

A variety of neurodevelopmental disabilities and mental illnesses have an increased risk in young male children that includes attention-deficit/ hyperactivity disorder (ADHD), autism spectrum disorder, mental sub-normality, dyslexia, stuttering, cerebral palsy (CP), and early-onset schizophrenia (see Table 41.2) [2, 9–43]. Nocturnal enuresis can be classified as a parasomnia and is seen in 3–5% of 12-year olds is for both males and females while less than 1% of 19-year-old males. Nocturnal enuresis is more common in males, while secondary enuresis is more common in females [3, 4].

Males tend to have a slight increase in narcolepsy versus females; narcolepsy-cataplexy has a prevalence of 0.02 to 0.04% [9]. Obstructive sleep apnea hypopnea occurs in 1–2% of children with similar male to female ratios in childhood and a somewhat increased risk of post-pubertal males; in adults the male to female ratio is 2:1 to 4:1 [9]. Sleep terrors are more common in male children [9]. The Klein-Levin syndrome (sleeping beauty syndrome) is typically reported in adolescent or young adult males [4].

Attention-Deficit/Hyperactivity Disorder (ADHD)

ADHD is found in 4–15.5% of the pediatric population and 2.5–5% of the adult population as noted by a variety of research studies [2, 9–14]. An increased risk is noted in males with 2:1 male to female ratio of ADHD in children and 1:6:1 ratio in adults [9]. Females have an increased risk of attention deficit disorder without hyperactivity versus males who have an increased risk of hyperactivity and ADHD [9, 15–17]. The influence of co-morbidities (i.e., autism spectrum disorders, conduct disorder, oppositional defiant disorder, anxiety disorder, others) on gender differences in ADHD remains under study [1–3, 9, 18, 19]. The influence of early adolescence on both male and females with ADHD remains under research as well [20].

Table 41.2 Neurodevelopmental disabilities with increased prevalence in males

Nocturnal enuresis
Attention-deficit/hyperactivity disorder
Autism spectrum disorder
Intellectual disability
Dyslexia
Stuttering
Cerebral palsy
Early-onset schizophrenia
Parkinson's disease (adult males) (23, 24)

Autism Spectrum Disorder (ASD)

The prevalence of autism spectrum disorder (ASD) with Diagnostic and statistical manual of mental disorders, 5th edition, american psychiatric association (DSM-5) criteria is approximately 1% and male to female ratio of 4:1 is typically noted; 2:1 in those with ASD and intellectual disability (moderate to severe) [1–3, 9, 21–24]. Causes of gender-influenced differences may, in part, be related to differences in microglia and their effects on inflammation modulation traced back to early brain development [24]. Various conditions can be co-morbid and associated with ASD that are also increased in males, such as ADHD, intellectual impairment, and developmental language disorder [1–3, 9]. Some genetic conditions can be seen in persons with ASD that have an increased risk in males such as the Fragile X syndrome and Klinefelter syndrome [21, 22]. Avoidant/restrictive food intake disorder can be comorbid with ASD and this combination in more common in males versus females [9].

Intellectual Disability (Intellectual Developmental Disorder)

The prevalence of the DSM-5 neurodevelopmental disorder, *Intellectual Disability)*, is approximately 1% of the general population that includes 6 per 1000 for severe intellectual disability [9]. Intellectual developmental disorder can also be viewed in a bell-shaped distribution in which it is noted in 2.5% of the general population [25]. Males are at increased risk of this disease category with a male to female ratio of 1.6:1 for mild disease versus 1.2:1 for severe intellectual disability [9, 21, 25, 26]. X-linked mental retardation syndromes are frequent and are mapped to gene loci on the X chromosome [26].

Causes for such gender disparity may lie, in part, to increased risk of the male brain to early (in utero) brain insults [9, 25]. Lending support for this risk to the male brain in early development is the co-morbidity of various neurodevelopmental disorders with increased prevalence in males—such as ADHD, ASD, and others [1–3, 9]. Males have an increased susceptibility to dopamine system deficits leading to increase in such conditions as ADHD, ASD, Parkinson's disease, and others [23]. Research is currently occurring in the Y-chromosome gene (SRY) and its activities in the male brain [23].

Specific Learning Disorder

Specific learning disorder that involves such domains as mathematics, writing, and reading are seen in 5 to 15% of school-aged children and is increased in males with a male to female ratio of 2–3:1 [9]. Reading disorder (dyslexia) preva-

lence varies in studies from 2 to 8% of school-aged children and is increased in males [27].

Communication Disorders

The DSM-5 classification of communication disorders includes language disorder, speech sound disorder, childhood-onset fluency disorder (Stuttering), and others [9]. Males and females are at equal risk of stuttering between ages 2 and 4; however, three times more boys than girls continue with abnormal fluency disorder and require management [28]. There can be associated with motor movements such as tics, head jerks, or tremors of the face [9]. The presence of sex chromosome aneuploidies increases the risk of specific language dysfunction as well as dyslexia [29].

Tourette's Disorder

Various research studies place the prevalence of Tourette's disorder in a range of 3–8 per 1000 children of school-age and a male to female ratio of 2–4:1 [9, 30]. ADHD is associated with Tourette's disorder and is also increased in males, as noted earlier in this discussion [31]. Underlying epigenetic etiologic factors are under study that includes looking at the significance of DNA methylation in TD [32].

Cerebral Palsy

Various studies report that cerebral palsy occurs in 1.5 to 2.5 per 1000 live births and a male to female ratio of 1.33–1.4:1 [2, 33–37]. Some data suggest a prevalence of 3.3 per 1000 live births and an increase due to survival of very low weight premature infants [35, 38]. Various conditions can be seen with or associated with CP including learning disorders and ADHD that are also increased in male children [34].

Schizophrenia

The lifetime prevalence of schizophrenia is 0.3–0.7% with a typical onset of the early to late twenties for males and late twenties for females; overall, there is a "slight" increase in males versus females with this adult-onset disorder [9]. Early-onset schizophrenia (EOS) refers to schizophrenia when psychotic symptoms emerge prior to 18 years of age; childhood-onset schizophrenia (COS) refers to situations in which schizophrenia-like symptoms develop under age 13 [39–41].

COS is noted in 0.04% (or less) of the population and represents 0.1–1% of all schizophrenia; a prevalence of 1 in 10,000 is suggested for EOS and 1–2 in 100,000 for COS [39–42]. Males have an increased risk of schizophrenia with a 1.4–2:1 male to female ratio; most studies note this risk for males, though the precise male to female ratio may vary from study to study [39–41]. An increased prevalence of schizophrenia in persons with intellectual and developmental disabilities has been noted in various studies [2, 43].

Neurodevelopmental Disabilities and Females

A variety of neurodevelopmental and neuropsychiatric conditions have increased prevalence in females, as noted in Table 41.3. Some of these conditions are briefly considered.

Myelomeningocele

Neural tube defects (NTDs) occur from failure of neural tube closure in early in utero development and the most severe type of NTD is myelomeningocele in which vertebral column defect allows protrusion of meninges and spinal cord at the level of the defect [2, 44–47]. NTD prevalence varies in studies in different parts of the globe (with the highest rates noted in Wales, Ireland, India, China) and such variability is due to both environmental and genetic factors [2, 44–47].

Myelomeningocele is found in 1 in 4000 live births in the United States and lumbar myelomeningocele, the most common type, is 3–7 times more common in females versus males [2]. The prevalence of NTDs has been reduced over the past several decades due to such factors as increased global use of folic acid supplementation during pregnancy, improved nutrition, and prenatal diagnosis of this condition [2, 44–47].

Mental Health Disorders

In contrast to males, pubertal females are at increased risk of certain mental health disorders that include eating disorders, anxiety, and depression [1, 3, 5, 9, 21, 23, 24, 48].

Table 41.3 Neurodevelopmental disabilities with increased prevalence in females

Myelomeningocele
Eating disorders (anorexia nervosa, bulimia nervosa)
Depressive disorders
Anxiety disorders
Multiple sclerosis
Others

Eating Disorders

The 12-month prevalence of anorexia nervosa is approximately 0.4% in young adolescent females and 1–1.5% for bulimia nervosa in young adolescent females [9]. A female to male ratio of 10:1 is reported for both anorexia nervosa and bulimia nervosa [9]. In binge-eating disorder, a 12-month prevalence of 1.5% is reported for adult females versus 0.8% for adult males [9]. Anxiety and depression disorders are co-morbid with these eating disorders [9, 48].

Depressive Disorders

Many studies note that depression (mood disorder) is common in adolescents and adults with an increased risk of females [1–3, 9, 21, 23, 24]. Studies typically conclude that depression is equal among females and males before puberty develops, while the advent of puberty increases depression in female adolescents—doubling or tripling the rate in contrast to males [49–52]. Females with depression have increased risks of co-morbid anxiety disorders and ADHD [52]. Persons with developmental disabilities are at increased risk of depression and anxiety [2]. Research in sex chromosome aneuploidies are seeking to provide more clarity in gender differences in such entities as depression and anxiety disorders [21].

A U.S National Health Interview Survey for parents of 7103 persons aged between 4 and 17 years from 2007 revealed that **3.0%** had been given a diagnosis of depression during the previous 12 months [50, 51]. In another parental survey (National Survey of Children's Health, 2007) of 78,042 participants aged 3–17 years of age, **3.9%** had ever been given a diagnosis of depression and **2.1%** had a depression diagnosis during the survey [50–52].

In a survey of 45,500 adolescents (aged 12–17 years) in the National Survey on Drug Use and Health (2010–2011), **12.8%** had a lifetime history of a major depressive episode; in this same survey, **8.1%** noted a major depressive episode during the 12 months prior to this survey [50, 51]. In a National Health and Nutritional Examination Survey of 2007 to 2010, 1782 youth aged 12–17 years surveyed, and **6.7%** noted a depression during a 2-week period just prior to this survey [50, 51].

Complicating epidemiologic data on depression is that many persons develop depressive symptoms that may not meet formal diagnostic criteria for a major depressive episode [9, 51]. Some research suggests that subthreshold depression has a point prevalence of 2.2–4.9% in those 13–16 years or age, a 12-month prevalence of 5.25–9.3% in those aged 11–17 years, and a lifetime prevalence of 6.3–22.9% for those 14–18 years of age [51]. Also complicating this picture is the reality of pediatric bipolar disorder that has a prevalence of 1 to 1.5% in the pediatric population; there is an increased risk in females and co-morbidity with eating disorders [9, 53].

Anxiety Disorders

The 2013 American Psychiatric Association DSM-5 lists over 20 anxiety disorders that include such entities as generalized anxiety disorder, obsessive-compulsive disorder, post-traumatic stress disorder, and others [9]. Prevalence studies on anxiety disorders in children and adolescents vary widely—ranging from 4 to 30% with an increased predilection for females in some anxiety disorders [54–57]. In epidemiologic studies, females account for two-thirds of general anxiety disorder [9].

Persons with anxiety disorders can have a number of co-morbid conditions including major depression disorder (most common), other mood disorders, language disorders, learning disorders, ADHD, oppositional defiant disorder, conduct disorder, and others [2, 54].

Other Conditions/Disorders

Females have an increased risk of insomnia with a female to male ratio of 1.44:1 that typically starts in young adulthood or earlier with the birth of the first child [9]. Sleepwalking is more common in female children versus male children, while eating during sleepwalking is more common in adolescent and adult females versus males [9]. Females are also more likely to have restless legs syndrome versus males (up to twice the rate) [9]. A differential diagnosis for autism spectrum disorder is the Rett syndrome that occurs almost always in female in early childhood.

Females have an increased prevalence for multiple sclerosis—a neurological and neurodegenerative condition mostly found in adults [24]. Adult women with a stroke tend to have less favorable outcomes than males with a stroke [24]. The role of microglial function and central nervous system neuroinflammation processes is under current research in this regard [24]. For example, there appears to be changes in inflammation processes with microglia early in brain development that may led to gender susceptibilities in multiple sclerosis, Parkinson's disease, ADHD, ASD, and other conditions [24].

However, one must be careful of data interpretation and repeat consistent data is needed to provide reassurance of specific gender differences. For example, the myth of female inferiority in mathematics skills has been debunked with early twenty-first century studies. The prevalence of dyscalculia varies in studies from 3 to 14% and research generally notes equal gender distribution though girls are decreased in advance math classes probably due to sociocultural and non-biologic or gender issues per se [58–61].

In Utero Milieu: Protection Versus Toxicity

The uterine-placental milieu can be a very protective environment that can safeguard the embryo-fetus and fend off various toxic as well as dangerous threats to the overall health of this development human being [62, 63]. The fetus has received various genetic factors from the biologic parents that combine with various positive and negative factors from the in utero-placental system—both internal and external to this amazing system. Some environmental exposures are known to be hazardous—such as seen with exposure to maternal infection, maternal trauma, malnutrition, mercury, lead, pesticides, tobacco, cannabis, alcohol, opioids, and other illicit drugs [62–72]. Exposure of the fetus to various forms of toxic stress can lead to injury to the early developing brain setting this person up for increased risk of developmental disabilities and other disorders in childhood, adolescence, and adulthood [62, 63, 73, 74].

Research over the past decades has led to the Barker hypothesis or fetal origins hypothesis in which conditions in pregnancy and during birth influence later health in animals and human beings [75]. Stress to the mother (i.e., infection, trauma, others) induces a neuroendocrine response (with hypothalamic corticotropin-releasing factor with resultant release of thyroid and corticosteroid hormones) and phenotypic expression in the offspring that has influenced vertebrate evolution over millennia of life [76]. The psychoneuroendocrine interplay in pregnancy has been shown to influence the development and health of the fetus and the fetus' post-birth life [77].

Changes in the fetal hypothalamic-pituitary-adrenal axis from maternal stress (such as prenatal alcohol exposure) leads to profound changes in the developing person in the womb and beyond that can have various gender or sex differences [78, 79]. Changes in maternal HPA can led to mood disorders in the post-partum mothers and neurodevelopmental as well as cardiometabolic alterations in the offspring that may include over-exposure to the glucocorticoids [80–82]. This can involve changes in DNA methylation at the gene level [83].

Less than ideal or optimal synaptic connections may arise with defects in epigenetic DNA methylation, failure of proper fatty tissue insulation for neurons, and other abnormalities in the complex central nervous system development in the fetus, newborn, infant, toddler, and other childhood stage. Research also notes that the female HPA axis can be vulnerable to prenatal stresses resulting in the female fetus having augmented HPA axis reactivity and resultant disease vulnerability due to fetal glucocorticoid overexposure (see Table 41.3) [84, 85].

Placental Milieu

The placenta is a transient yet key structure in providing protection or damage to the growing fetus as it controls fetal exposure to the maternal and outside milieu; this involves cytokines, growth factors, endocrine factors, various nutrients, and others [86]. Adaptive reactions of the placenta to maternal stresses (with hypoxia, oxidative stress, intrauterine growth retardation, others) play an important role in positive as well as negative effects on the fetus for in utero life and post-birth life for the offspring [87].

Regulation of placental function by imprinted genes is in active study as placental efficiency and health influences both the fetus and the later adult with health or dysfunction [88–90]. Placental control of nutrients to the fetus is under epigenetic complex control of such chemicals as the peroxisome proliferator-activated receptors (PPARs) that influence the placental developmental plasticity [91].

Maternal inflammation and other stressors can alter placental output of serotonin (5-hydroxytryptamine or 5-HT) to the fetal brain (fetal forebrain) with potential effects on the mental health of this offspring—such as increased risks of autism spectrum disorder [92–94]. Indeed, the serotonin system in ASD is under active research which has included such observations as that hyperserotonemia was the first biomarker discovered in those with ASD—being found in 25% or children with this condition [95]. Various in utero factors (epigenetic and other genetic issues) are under research to determine sex programing causes in ASD and other neurodevelopmental disorders [96–99].

Epigenetic Role of O-Linked *N*-Acetylglucosamine Transferase (OGT)

As research continues to identify the fetus and child's epigenetic responses to various environmental and genetic stresses as well as toxicities, the question arises regarding reasons for certain *gender* vulnerabilities and susceptibilities that have been identified in this discussion. One of the key influences noted by research includes the master epigenetic role of O-linked *N*-acetylglucosamine transferase (OGT) that was identified from the early 1980s.

Stress during pregnancy has increased negative effects on the male fetus versus female fetus. There are differences in gene expression in both sexes due to the action of a molecule called OGT (O-linked *N*-acetylglucosamine transferase) which, when placed with the genome, changes gene responses via epigenetics involving modification of a protein histone H3 that is called H3K27me3 that can induce repression of various genes [5–8]. There is trimethylation of lysine 27 on the end (terminal) tail of the core histrone H3.

Research notes that high amounts or levels of H3K27me3 in the placenta, often found in the female fetus, allow the female fetus to become more resistant to maternal stress (i.e., from drugs, toxins, infection) [5–8]. The OGT gene is found on the X chromosome with resultant protection for the female versus male fetus. In Professor TL Bale's research and that of others (including animal studies), this leads to more autism and schizophrenia in the male versus female due to more effects on brain development [5–8, 99–114].

Conclusions

Neurodevelopmental disabilities exert a considerable amount of Daedalian difficulties for children, adolescents, young adults, and their families. These issues can complicate their adulthoods and require clinicians to be knowledgeable about these conditions. One of the perplexing problems in this regard is the role of gender and why some conditions are more common in one sex versus another (see Tables 41.2 and 41.3).

Some disorders are seen in the pediatric population, while others are more common in adulthood—such as Parkinson's disease, multiple sclerosis, and others. Research is focusing on the utero-placental unit and its ability to protect the fetus from maternal stress from such threats as infection, drug use, trauma, and others. The epigenetic role of O-linked *N*-acetylglucosamine transference (OGT) and other biochemical factors is under active research in establishing the vulnerabilities of the male versus the female gender.

Multiple Choice Questions

1. In contrast to males, females have an increased risk for:
   (a) Cerebral palsy
   (b) Autism spectrum disorder
   (c) Attention-deficit/hyperactivity disorder
   (d) Myelomeningocele
   Answer: (d)
2. In contrast to females, males have an increased risk for:
   (a) Multiple sclerosis
   (b) Anxiety disorders
   (c) Dyslexia
   (d) Depressive disorders
   Answer: (c)
3. The Kleine-Levin syndrome (Sleeping Beauty Syndrome) is typically identified in which group:
   (a) Preschool females
   (b) Latency age males
   (c) Adolescent and young adult females
   (d) Adolescent and young adult males
   Answer: (d)
4. In contrast to females, males have increased risks for:
   (a) Rett syndrome
   (b) Early-onset schizophrenia
   (c) Sleepwalking
   (d) Restless legs syndrome
   Answer: (b)
5. Parkinsonism is increased in which group:
   (a) Adult males
   (b) Adolescent males
   (c) Adult females
   (d) Adolescent females
   Answer: (a)
6. Multiple sclerosis is increased in which group:
   a. Adult males
   b. Adolescent males
   c. Adult females
   d. Adolescent females
   Answer: (c)

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A Non-Categorical Approach to Childhood Neurodisability: Concepts, Evidence, and Implications for Clinical Practice, Organization of Services, Teaching, and Research

Anton R. Miller, Emily Gardiner, and Peter L. Rosenbaum

Learning Objectives

1. Understand conceptual underpinnings of the non-categorical approach (NCA) to chronic health conditions of children and youth.
2. Be able to explain the special relevance of the NCA to childhood neurodisability, including neurodevelopmental pediatric practice and research.
3. Appreciate the relevance of the International Classification of Functioning, Disability, and Health (ICF) to both childhood neurodisability and NCA.
4. Familiarity with empirical evidence that supports the relevance and appropriateness of NCA in childhood neurodisability.
5. Appreciate the importance of adopting a NCA in childhood neurodisability, including planning and organization of clinical programs and services, health education, and research.

Highlights

1. The non-categorical approach (NCA) to chronic health conditions of children and youth emphasizes *commonalities* in the impact of discrete health conditions on the daily lived experience of child and family, and on implications for child and family needs.
2. NCA is particularly relevant to children with neurodisability and their families are given clinical heterogeneity within diagnostic categories; sharing of clinical phenomenology across neurodevelopmental conditions; and documented commonalities in terms of impact and need across condition types.
3. Furthermore, the traditional biomedical categorical approach based on "diagnose-and-treat" is less salient in the context of childhood neurodisability. Interventions for this population rarely target well-understood pathophysiologic processes; rather, they usually aim to mitigate effects of biological derangements and environmental adversities and to promote optimal child and family functioning.
4. NCA dovetails with the International Classification of Functioning, Disability, and Health (ICF) as ICF has a major focus on lived daily experience in the area of individual functioning, and on the personal and environmental factors that affect functioning.

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Introduction

You are sitting in the cafeteria at an empty table, eating lunch, when a colleague you recognize but don't know very well asks whether they can join you. You chat about what they do, and they tell you that they work with children with 'Developmental Condition X', and proceed to explain that these kids and families have 'the most complicated lives imaginable'. Another clinician

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then sits down and tells the same story about the challenges faced by kids and families in their clinical services for kids with 'Developmental Condition Y'. Finally, the fourth chair is filled by yet another colleague working with families with 'Developmental Condition Z', who tells a similar story. To the authors of this essay, these are people who don't get out enough—and have not been able to see the woods for the trees!

The authors of this chapter start from a view that neurodevelopmental pediatrics (NDP) is that branch of child health that explicitly addresses the *impact* of "neurodisabilities" on children with these child-onset conditions, and on their families. According to this approach, the point of departure for service providers and programs for this population of young people and their families is the acknowledgment that there are impairments in children's health or functional status that put the child's (and family's) developmental trajectory at risk. While efforts at specific diagnosis are important, as discussed below, professionals in this field are motivated especially, and perhaps primarily, by the predicaments of the children and families, more so than by diagnosis-specific considerations. While not challenging the value of a biomedical understanding of children's issues, this chapter presents that case—conceptual arguments and evidence—to support this expanded view of childhood disability, a view that moves beyond the biomedical to consider life-course perspectives. In this chapter, we explain how this view overlaps with, and derives some of its power from, a long-standing yet still underutilized approach to childhood neurodisability known as "the non-categorical approach."

Background Concepts

Much of neurodevelopmental pediatrics (NDP) has traditionally followed the conceptual model underlying the practice of pediatrics, and of medicine generally, which is guided by a biomedically-grounded paradigm of diagnose-and-treat. We have argued previously [1] that this pathway may be most appropriate and effective with health conditions where the etiology and pathophysiology are well understood. Treatments and interventions for children with "neurodisability," a consensus-agreed term to describe the population that is the focus of NDP [2], are almost always less condition-specific than many treatments for acute-onset conditions. For example, in assessing and managing sudden chest pain, the diagnosis-to-treatment paradigm may literally be life-saving. In NDP, treatments and interventions are less aimed at halting or reversing known biomedical derangements than they are at promoting functioning [3]. A modern disability-grounded perspective places individual functioning and its contextual determinants at the center of the discussion; this is consistent with the World Health Organization's International Classification of Functioning, Disability and Health (ICF) framework [4], and seems to the authors of this essay to be a far better fit for this population of children and youth and their families.

ICF emphasizes the description of functioning at various levels—the person's impairments at the level of "body structure and function"; the person as agent of daily "activities"; and the person "participating" as a social being. It does so alongside an accounting of the "environmental" and "personal" (so-called contextual) factors that so critically shape everyone's functioning. It is this emphasis on individual functioning that makes the "disability perspective" [1], based on the ICF concepts, dovetail with a non-categorical approach to chronic health conditions among children. In this chapter, we briefly review the origins of the non-categorical concept in child health and explain its special relevance for the population of children with neurodevelopmental conditions and their families. We advocate for wider implementation of this approach/perspective in the provision of clinical and supportive services for these children and their families; for the organization and delivery of community-based services; for policy-making at government levels; and for education of health professionals and for research involving this population.

The Non-categorical Approach: Overview

Diagnoses are categories. Our clinical investigative efforts strive to help us know whether it's "this" or it's "that" condition. The idea of a "non-categorical" approach was pioneered by visionary thinkers in community and population child health in the USA and the UK in the 1970s and 80s, most notably by Bob Haggerty, Barry Pless, Philip Pinkerton, and Ruth Stein. They drew attention to both the rising prevalence and salience of chronic health conditions among children, noting that chronic health conditions, when taken collectively, accounted for a considerable burden of morbidity among children and their families, even though specific individual conditions were often uncommon [5]. They argued, and brought forward evidence to support their contention, that the needs of children affected by chronic conditions, and the needs of their families, were not well addressed within a health services system (in reality, a disease-care system) designed to treat, and ideally to cure, acute episodic illnesses, most of which had well-understood etiologies. Rather, they recognized the many *commonalities* that exist across a range of specific chronic conditions, and proposed that instead of the traditional focus on the diagnosed condition as the "entity-of-interest," these commonalities should be placed at the forefront when planning services and supports, and given more prominence in educational and research agendas. They captured the essence of the non-categorical approach in this way: "The chronicity of the illness [today we would use the term 'condition'] and the impact it has on the child, his par-

ent, and his siblings, is more significant than the specific character of the disorder…there are certain problems common to all chronic illness over and above particular challenges posed by individual needs" [6].

These commonalities are largely viewed in terms of the *impact* of the condition on child and family, with direct relevance to the *needs* that children and families have for health and other developmental and community-directed (e.g., educational, recreational, and vocational) services within a broad holistic conceptualization. This set of considerations includes children's physical, mental and developmental health, and the health and well-being of the child's family. Impacts on children can include effects on attendance at school, learning, and social-emotional development; impacts on families can include effects on mental and physical health and on economic well-being, related to the special additional practical and emotional demands of their child's "complicated life." This approach increases the salience of chronic conditions as a collective group: it makes the conditions and their impact on society more visible, and it leverages their claim for prioritization in policy-making at the population level. At the same time, the approach helps individual children and families to cope and thrive when key interventions such as (re)habilitative (we prefer "developmental") services are directed toward optimizing functional skills and participation, or supporting family coping and well-being.

A non-categorical approach often comprises services and supports that cannot be defended as diagnosis-specific, because they are, in essence, generic; they transcend specific conditions and address functional challenges. Technologies such as wheelchairs are not specific for people with cerebral palsy (CP); and gastrostomy feeding tubes have been helpful for children with a variety of neurodevelopmentally-based feeding disorders. The same applies to many of the medications used in NDP. For example, psychostimulants are primarily indicated for attention deficit hyperactivity disorder (ADHD), but may also be useful in patients with decreased level of consciousness post-brain injury. Similarly, "antidepressant" drugs of the selective serotonin reuptake inhibitor type may be helpful in the management not only of depression, but also of anxiety or rigid and obsessive behavior patterns that may occur in children diagnosed with autism spectrum disorder (ASD), as well as other genetic and acquired conditions; and baclofen can be helpful in the management of spasticity in the context of CP and of other neurological disorders. A non-categorical approach also serves to shift focus to the *lived experience* of the child (and family) rather than on a *disorder* or disease. Planning and providing services and supports can be done more efficiently and effectively when organized around child and family needs—which almost always cut across discrete condition types—than when services are organized on a condition-by-condition basis. By focusing on the *impact* that a health condition is having on that child in that family, the non-categorical approach is a more rational, as well as a fairer and more just (ethical) way to determine access to resources, compared to diagnosis-based eligibility as has been the tradition in health care.

The Case for a Non-categorical Approach in Childhood Neurodisability

The earliest work on non-categorical or "generic" approaches was aimed at studying psychosocial correlates of chronic health conditions (see Pless et al. 2010 for a helpful historical review and summary) [7]. The development of operational definitions for the population, and the creation of appropriate measures for use in research and policy discussions, took a broad view of chronic health conditions ranging from CP to cystic fibrosis [8, 9]. Later on, terminology expanded to link chronic health conditions with disabilities [10]. This came about in part because the framework proposed to identify children with chronic conditions recognized fundamental aspects of these conditions to be chronicity, along with three classes of sequelae or consequences that make having a chronic condition salient: (a) limitation of function, activities or social role; (b) dependency on interventions like medications, technologies or personal assistance to compensate for or minimize such limitations; and (c) the need for medical care or related services, or psychological or educational services beyond what is usual in traditional diseased-focused biomedical services [9]. All of these aspects are, to a greater or lesser extent, part and parcel of the realities of daily life for children with neurodisability and for their families. But for children with neurodisability, research, policy-making and service delivery and organization continue to have a strong categorical underpinning, as reflected in the way services are funded and provided, the continuing creation of policies by "disease" category, and the insistence among funding agencies that research in this field be undertaken about specific conditions.

While the original non-categorical population proposed by Pless, Stein, and others was designated as "children with chronic health conditions," the term "children with special health care needs" rose to prominence as a non-categorical formulation that achieved considerable traction with policymakers, service planners, and researchers in the USA from the late 1990s onwards [11, 12]. More recently, the designation "children with medical complexity" has emerged as an increasingly salient non-categorical population [13].

Although children with neurodisability exist as a subpopulation within all these other non-categorical populations, there are compelling grounds to argue that children with neurodisability comprise a particular non-categorical population that warrants more explicit recognition and attention. First, in keeping with something that early proponents of the non-categorical approach recognized for chronic health condi-

tions in general, individual conditions within childhood neurodisability may be uncommon or rare, but collectively these children account for between 7 and 14% (depending on definition) of all children in developed societies [14–16]. Second, children with neurodisability and their families share not only many *needs* across discrete neurodevelopmental conditions, arising as a consequence of these conditions, but also many *clinical features,* given the dual phenomena of symptom heterogeneity and comorbidity or complexity of clinical presentation. Indeed, the very substrate of childhood neurodisability—dysfunction in brain pathways underpinning developmental functions—makes a traditional categorical approach, based on Diagnostic and Statistical Manual of Mental Disorders (DSM) or International Classification of Diseases (ICD) diagnoses, limited and questionable as a vehicle to help affected children and families.

In terms of sharing of needs across discrete neurodevelopmental conditions, the presence of neurodisability carries implications for services and supports that a child and family will need in order to optimize functioning and to navigate and cope with educational, social, and recreational experiences. It also comes with important implications for how the child's family supports optimal development for their child while coping with caregiving demands and challenges that go beyond what most parents deal with (and what therefore make their lives "complicated"). Crucially, for children with neurodisability, there is a great deal of commonality in these implications or consequences of neurodevelopmental disorders and disabilities *across* condition-types. Though a child's condition may lead, for example, to a *primary* impact on mobility, or communication, or learning, most children with neurodisability present with differences and needs across multiple domains of developmental functioning, a reality that is not necessarily the case for children with more "medical" or non-neurodevelopmental chronic conditions or special health care needs such as asthma or diabetes. Another common thread in the experience of children growing up with a neurodisability is the likelihood of some element of "deprivation" based on the restrictions on experience that may be imposed by the primary impairment. (Think, for example, of how restrictions in mobility, or vision, or communication may fundamentally impact a child's early experience of their world—and of other people's views of that child.) The focus on functional limitations and strengths, participation in daily activities, both domestic and social, and the contextual factors that promote or hinder participation takes us beyond what a child's diagnosis can (and cannot) tell [9, 17], and squarely into non-categorical territory.

In terms of clinical features, it is increasingly clear that children with neurodisability are a complex and highly varied population whose real-world clinical presentations only occasionally reflect the clean descriptions of neurodevelopmental disorders set out in the manuals where these conditions are typically classified. Heterogeneity of symptoms *within* conditions is a known attribute of categorical-polythetic diagnostic classification systems, as are used in the DSM taxonomy [18]; within these systems, symptom profiles can vary markedly across individuals who all meet criteria for membership of a diagnostic category [1]. Clinical heterogeneity has led to more widespread acceptance of the notion that conditions like ASD and CP are not, in fact, unitary conditions, and to new nomenclatures such as "the autisms" [19], and the terminology of spectrum disorders as applied to cerebral palsies [20], autistic disorders, and neurodevelopmental disorder following alcohol exposure, widely known as fetal alcohol spectrum disorder (FASD). In tandem with this, comorbidity and complexity of presentation have emerged as a hallmark of children in this population [21–23].

In the next section, we seek to buttress the case for non-categorical thinking in NDP by going beyond broad principles and concepts to a review of relevant evidence. But before that, we close this section with an important disclaimer: we are *not* arguing that diagnosis and diagnostic categories have no place in NDP. Having a diagnosis is of unquestioned importance in the lives of families, and often of patients. Knowing what "it is" (and what it is not) allows for a sense of identification and affiliation with other people, affords ways to think, talk, and act about their child, and to know what to pay attention to. In addition, some neurodisabilities have clear and proximate genetic underpinnings, the understanding of which can be very helpful to families with respect to recurrence risks and family planning. Uncovering the causal pathways for specific disorders (examples include maternal Rh incompatibility causing kernicterus and athetoid CP, and maternal iodine deficiency causing cretinism) has led to amazing preventive efforts for these specific kinds of CP. Within a disorder category, also, there may be more or less strong evidence regarding prognosis for that condition (taking account of the individuality of that child's specific manifestations of the condition). At an epidemiological level, diagnostic precision is required to count specific conditions and track a condition's incidence and prevalence. And finally, the classification of people by diagnosis is of unparalleled convenience for multiple administrative purposes which we have discussed elsewhere [1]. As such, there are many ways in which diagnosing and diagnoses are important. What we are arguing for in this chapter is a better balance of non-categorical and categorical thinking in the field of child health known as NDP; the conceptual base is compellingly relevant, and there is a solid body of supportive research evidence.

The Non-categorical Approach: State of the Evidence

Support for the validity of a non-categorical (i.e., function-based) perspective, in contrast to one that emphasizes the child's specific disorder, comes from three main strands of

research: (i) demonstrations of lack of specificity in the phenomenology of diagnostic categories in widespread use; (ii) demonstrations of commonality of experience and need across neurodevelopmental diagnostic categories; and (iii) studies that have directly compared the utility and relevance of functional aspects of the child's health (non-categorical ideas) to diagnostic status (categorical approaches).

When we think about the way in which children with neurodisabilities present, we categorize their abilities and challenges across the classic developmental domains, such as socialization, communication, self-care, cognition, behavior, and motor skills [24]; children are then classified into disability categories based on their (assumed) "primary" domains of impairment. For example, the DSM-5 [18] delineates individuals with ASD by their hallmark social-communication difficulties, whereas children with intellectual disability (ID) are distinguished by cognitive-adaptive challenges. In reality, however, the nature of children's functional impairments does not follow such clean, distinct boundaries; although each disorder is characterized by shared core symptomatology, affected children experience challenges that permeate the boundaries of each developmental domain.

Examples of this abound in the literature. Approximately 60% of individuals with ASD also present with an intellectual disability or are in the borderline range (IQ 71–85) [25], and many experience significant challenges with adaptive functioning, regardless of intellectual ability [26]. Similarly, children with FASD, ADHD, and tic disorders experience social-communication challenges [27–29]. Across studies, between 23 and 65% of children diagnosed with ASD also have attention problems or ADHD [30, 31]; significant impairment in motor coordination skills is also highly prevalent among children with ASD [32, 33].

Furthermore, 46% of children with ADHD have learning or language disorders [34]. These and other findings force us to confront the following questions. Given this clinical heterogeneity, how useful it is to retain a fixed attachment to a categorical way of thinking about the health and needs of children in this population? And which category should have primacy when there is a diagnostic "salad" as described above? Yet all too often, the child's "diagnosis" tends to remain the primary approach to identifying the child and their needs in the minds of clinicians, parents, educators, and social services administrators.

In an early and seminal study of functional characteristics in relation to chronic condition type, Stein and Jessop (1989) [17] reported greater functional heterogeneity *within*, rather than *between*, a number of chronic conditions (asthma, meningomyelocele, hydrocephalus, seizure disorders, and hemoglobinopathies), and failed to find significant differences across diagnoses on child functional status, behavior problems, mothers' psychiatric symptoms, or family impact. This research provided early empirical evidence that, regardless of specific diagnostically-labeled condition, there are important shared aspects of the affected individual and family's experience (i.e., the experience of functional challenges or disability).

At the time, Stein and Jessop (1989) [17] suggested that their conclusion—that diagnosis provided little meaningful information for important child and family outcomes—was likely "obvious to many social scientists" (p. 773). Despite their seemingly self-evident conclusion, however, nearly three decades later the literature is still dominated by research adopting the condition-specific lens, comparing children within commonly-used diagnostic categories on various indices of functioning. The rationale for this condition-specific, categorical approach is that it provides insight into which children may be at heightened risk for particular functional difficulties by virtue of their diagnosis; the findings can then illuminate targets for (often-limited) social services supports, which would be directed towards the "most-burdened" families. The pitfall of this diagnosis-specific categorical approach, however, is that the very significant heterogeneity characterizing children *within* each category [21, 22] is masked by mean scores [35]. This leads to potentially artificial generalizations about, for example, "children with ASD" as a group (a condition often highlighted as being associated with severe and pervasive behavior problems [36, 37]), as compared to those with Down syndrome (often highlighted as being "advantaged" by fewer behavior problems and high prosociality [38, 39]). In reality, many children with ASD may demonstrate relatively few behavior difficulties, while some children with Down syndrome may exhibit severe internalizing and externalizing challenges. Therefore, advancing sweeping recommendations about support-related needs, based on generalizations from this categorically-oriented work, is somewhat concerning, as children are excluded from consideration solely on the basis of diagnosis as opposed to functional need [1, 40, 41], and the individuality of their needs is lost.

Research on behavior problems serves as a prime exemplar of the predominant categorical approach to examining functioning amongst children with neurodisability—and the limitations of this thinking. Perhaps due to their pervasiveness [42, 43] and particularly impactful nature [44–50], this is one of the most researched domains of functioning. Within this work, children with ASD are typically highlighted as being most at risk in terms of severity (e.g., [36, 37]), yet research seeking to elucidate potential cross-condition similarities paints a more nuanced picture. Russell and colleagues (2013) [51], for example, compared children with ASD and ADHD on conduct problems, emotional symptoms, hyperactivity-inattention, peer problems, and prosociality, observing substantial overlap in both severity and impact, as rated by parents and teachers.

Work by the authors of this chapter further disentangles what can be gleaned from the mean difference versus overlap approaches. When comparing children with ASD, global developmental delay/intellectual disability (GDD/ID) and CP on mean scores alone, children with ASD were found to have significantly elevated conduct, hyperactivity-inattention, and peer problems as compared to those with CP, but only the latter challenge was elevated in comparison to those with GDD/ID (no differences were found across groups on emotional symptoms) [52]. Despite these differences, substantial overlap was clearly observed when the full distribution of scores was plotted for each group, particularly across those with ASD and GDD/ID on conduct problems, hyperactivity-inattention, and peer problems, as well as across all groups on emotional symptoms (see Fig. 42.1). Finally, children within each condition demonstrated matching patterns of peaks and valleys in terms of behavior problem type, with hyperactivity-inattention difficulties rated as most severe and conduct problems the least (see Fig. 42.2).

In another study by our group, descriptive data were extracted from clinicians' reports in order to determine whether there was overlap in the kinds of behavioral and emotional concerns observed across children being assessed in three distinct clinics at a developmental and rehabilitation service center [53]. Three concern clusters (from a possible 28)—namely "Tantrums/Outbursts/Meltdowns," "Inflexibility/Gets Stuck or Fixated," and "Social Behaviors"—were among the most frequently reported, regardless of clinic. Collectively, the highlighted research suggests that behavior problems, a significant functional characteristic of children with neurodisabilities, are shared quite widely across conditions. This research highlights but one functional domain; yet, the broader literature also provides evidence for similarities across individuals with various conditions in terms of genotype [54], symptomatology [55], brain circuitry [56], and comorbidity profiles [57]. This body of work reminds us to temper our ingrained expectations that children with particular conditions will present with distinct profiles and even that there are unique brain- and genetically-based mechanisms at work. Indeed, there is increasing biological evidence from genetic studies that *etiologies* of neurodevelopmental conditions such as ASD, ADHD, and ID are not distinct [58]. All of these findings and evidence call into question the ways in which we have traditionally delivered services, established support-related policy, and framed our research questions, and beg the question: Have we been too narrow in our approaches?

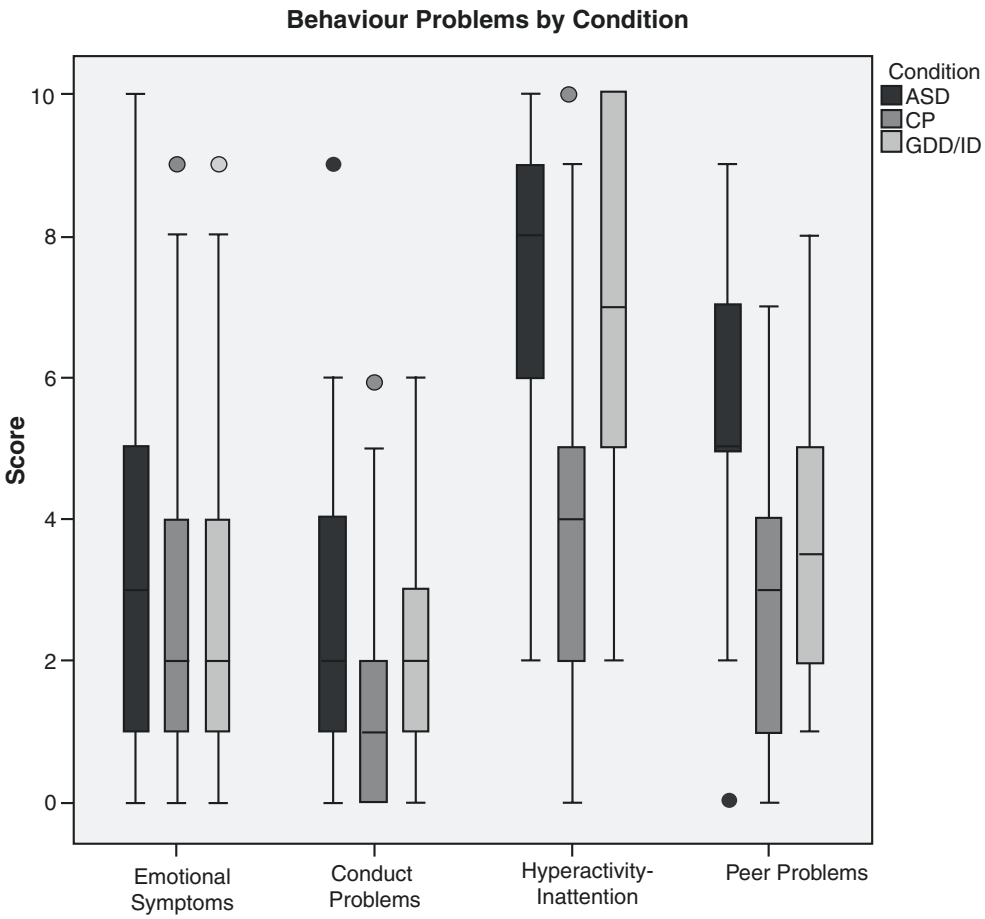

Fig. 42.1 Box plots for behavior problems across conditions. (With permission from John Wiley and Sons Ltd. [© 2019 John Wiley & Sons Ltd]. Gardiner, E, Miller, AR, Lach, LM. Topography of behavior problems among children with neurodevelopmental conditions: Profile differences and overlaps. *Child Care Health Dev*. 2020; 46: 149–153. https://doi.org/10.1111/cch.12720)

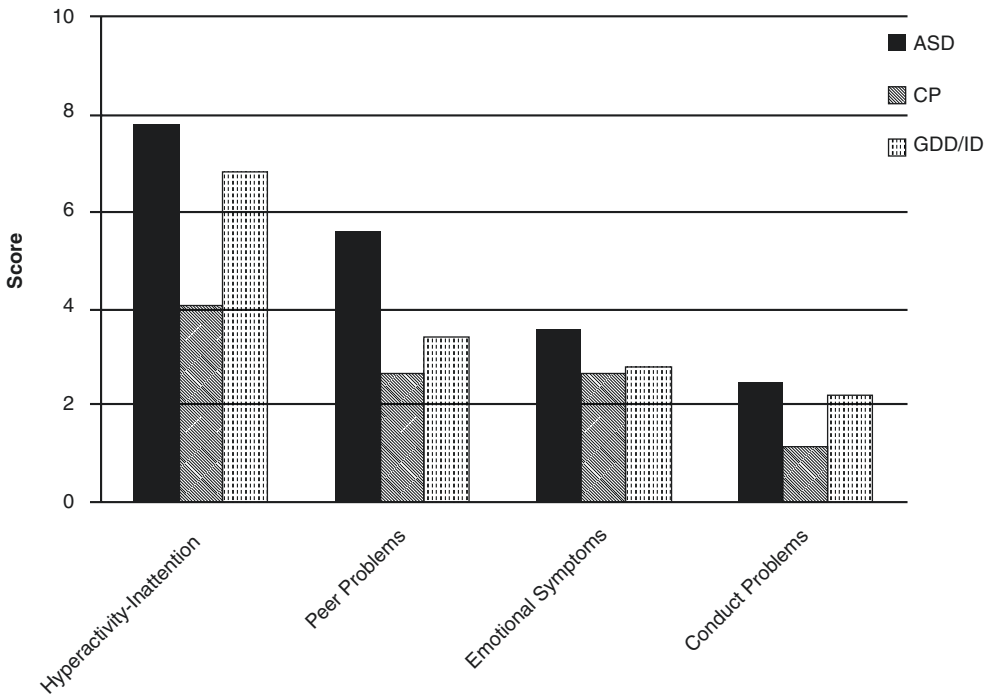

Fig. 42.2 Behavior problem profiles across conditions. (With permission from John Wiley and Sons Ltd. [© 2019 John Wiley & Sons Ltd]. Gardiner, E, Miller, AR, Lach, LM. Topography of behavior problems among children with neurodevelopmental conditions: Profile differences and overlaps. *Child Care Health Dev*. 2020; 46: 149–153. https://doi.org/10.1111/cch.12720)

With regard to practice, the evidence in support of the non-categorical perspective suggests that interventions developed for functional challenges within specific populations (e.g., behavior problems among those with ASD) can be applied more widely, thereby benefitting potentially underserved children and families. This is true at least in part because, despite arguments to the contrary, virtually none of our behavioral or psychosocial interventions is condition-specific. Indeed, in the stream of research that involves direct comparisons of utility and relevance of functional aspects of the child's health versus diagnostic status, it is children's functional characteristics (particularly activities of daily living), and not their specific health conditions, that best predict service need, both perceived [44] and awarded [59]. Moreover, there is evidence that many of the therapies accessed within such a system are applicable across the range of disability and can be appropriately adapted based on profiles of functional strength and challenge (e.g., applied behavior analysis, positive behavior support, cognitive behavior therapy) [60–62].

Further direct evidence is found within research demonstrating that diagnosis is not the best predictor of healthy adaptation among children with neurodisabilities and their families. With regard to child participation, research demonstrates that functional indicators, such as difficulties with cognition, speech, emotion, behavior, and self-care, have been found to account more fully for child participation both in school (playing with others, classroom participation, school outings and absences) and in the community (leisure and social activities, including involvement with music, sports, and summer camps) than does diagnosis [24, 63]. These findings also align with research indicating that it is not a disorder's "hallmark" impairments (e.g., social-communication skills among those with ASD or gross motor function in children with CP) that are most meaningful for various proximal and long-term outcomes. For example, adaptive, as opposed to social, functioning has been identified as most predictive for future social adaptation and independent living status (i.e., work status, residential situation, number and quality of friendships) among individuals with ASD [64], as well as for family outcomes, including perceived negative impact and quality of life, for those with ASD, ID, and CP [45, 65]. As described, the central premise of the non-categorical approach is that a diagnostic label does not act as a blueprint for how the child will function and behave, for their perceptions of personal health and well-being, or for how well they are able to participate across daily contexts. These, as Ronen and Rosenbaum (2013) [66] describe, reflect more comprehensive and individualized indicators of "health" (or "better health outcomes") than do purely biomedical features of disease.

Implications of These Ideas

The authors of this essay strongly believe that—without sacrificing any of the potential value of making specific diagnoses in children and youth with neurodisabilities—it is time to revisit how we think, talk and act with respect to the non-categorical approach to the issues in our field. If we are to

move the field forward, we need to adopt these ideas, and to model them for both families and learners. Let us consider how these lessons can be applied in the clinic, in our programs, in policy-making, and in health services education and research.

In the clinic, regardless of whatever approach we take, we know that we need to individualize our services to each child and family by being child-and-family-focused, and problem-focused, rather than relying solely on the diagnosis to guide an approach to management. As has been argued and illustrated in this chapter, developmental and behavioral issues—be they intrapersonal matters, behavioral challenges, or limitations in functional domains such as communication, activities of daily living, and mobility—need to be seen through a lens of *functioning* and not simply as manifestations of a specific disorder. This is important both because, as we have argued, virtually none of our developmental interventions is diagnosis-specific, and because interventions need to be tailored to the specific strengths and challenges faced by *this* child and *this* family at *this* time.

The corollary to this idea is that our programs and services can—we believe must—be structured around common functional approaches to problems and no longer solely around diagnostic labels. The huge advantages of such an approach should include economies of scale and the wider availability of services related to common problems rather than to diagnosis-specific "therapies" with long waiting lists in many "popular" programs. It also means that instead of waiting for all the assessment and test results to come back to guide a condition-specific treatment (which we believe to be few in number, if any), interventions and advice to parents and families can be offered quickly while diagnostic efforts are being pursued in parallel. The stepped diagnosis/stepped care approach, in which the question of diagnosis is revisited over time *pari passu* with interventions provided to young children and their families [67–69], is an example of this model of care. These and other non-categorical approaches in no way obviate the need for expertise in particular condition-specific issues, for example in self-injurious behaviors; however, as argued elsewhere in this chapter, we see services like these addressing functional matters rather than assuming that these problems are diagnosis-specific.

There are important implications of these ideas for policy-makers—and for clinical leaders who have (or need to have) their ear. Those of us who advocate for these (or indeed any) evidence-based approaches to services need to inform our policy-making colleagues about these alternate ways of thinking. Policy-makers are smart, dedicated, and hard-working professionals, but they are not likely to be up to date with emerging approaches unless we bring them these ideas and the evidence to support the arguments. That means that leaders in the field must be "Knowledge Brokers" who accept the challenge to inform others and advocate for ideas such as the ones proposed in this chapter.

With respect to health services research and the traditional conservatism of many funding agencies (and grant reviewers, who are often specialists in particular conditions), we cannot simply take for granted that people understand and accept the non-categorical thinking that we value so strongly. It is up to us as researchers, without being angry or challenging, to make clear arguments in support of the non-categorical thinking so that others can appreciate the value of exploring many issues *across* disorders and conditions. One of the obvious values for research to take this broader non-categorical approach is the potential to be able to increase sample sizes and achieve what is often very difficult to do in condition-specific research, especially with rare conditions. To address potential worries by granting agencies, we can of course indicate that we will analyze the findings of our studies both in the aggregate and by diagnosis or "category."

We strongly believe that the concepts discussed in this chapter must become part of the educational experience of health professionals across all our disciplines. This set of ideas is *complementary* to—and an expansion on—the traditional biomedical (diagnostic/categorical) ways that health professionals are taught. We have tried to argue herein, and would emphasize this point with all learners, that there can (must) be an awareness of the nature of people's individual "predicaments" within their "diagnoses"—and that personalizing our assessments and interventions to address the *functional* impacts of people's conditions in no way devalues other considerations or approaches.

Finally, we draw readers' attention to some work by one of the authors, whose embrace and operationalization of the WHO's ICF led to the creation of the "F-words" in childhood disability [3]. This whimsical set of ideas reimagines the WHO ICF framework of "body structure and function," "activity," participation," "environmental factors" and "personal factors" by considering and emphasizing these elements of health as "Fitness," "Function," "Friends," "Family," and "Fun," respectively. By adding the element of "Future," this completely non-categorical set of ideas allows families and service providers to create a holistic view of an individual's life, and importantly, to do so with a framework from which to set context-specific and individualized goals. These concepts are important to all individuals, not just to those with neurodevelopmental differences. The evidence is clear that families and colleagues around the world are embracing the F-words concepts regardless of condition, language, or location. (Readers seeking more detail about this application of a non-categorical approach can visit www.canchild.ca/f-words.)

Concluding Comments

We conclude by returning to the scenario sketched out at the opening of this chapter—clinicians involved in NDP who get together in the cafeteria, each claiming that their categorically-defined population is the most complex. Wider awareness and wider adoption of a non-categorical approach in NDP can help to move beyond this blinkers-on approach and get to the real needs of children and family affected by neurodisability. We hope our review of concepts and evidence of heterogeneity *within* diagnostic categories and commonalities of experience and need *across* categories has demonstrated the limitations of placing diagnostic categories at the center of organization and provision of services and supports, and the value of a more functionally-based, non-categorical perspective. The ideas presented in this chapter would enable all four lunch-mates to have common frameworks and concepts with which to share ideas and to collaborate as clinicians, teachers, researchers, and influencers!

Multiple Choice Questions

1. The non-categorical approach in child health focuses on:
   (a) Chronic health conditions
   (b) Diagnosis of discrete health conditions
   (c) Assignment of child and family supports based on functional need(s)
   (d) Assignment of child and family supports based on the child's diagnosed condition
      1. (a) and (c)
      2. (b) and (d)
      3. (a), (b), (c)
      4. All of the above
      5. None of the above

   ANSWER: 1
2. Core components of the non-categorical approach to child health are:
   (a) Biomedical diagnosis-to-treatment pathway
   (b) Recognizing commonalities in impact of condition across discrete conditions
   (c) Dismissal of the significance of medical diagnoses in child health
   (d) Identifying commonalities in child and family need across discrete conditions
      1. (a) and (c)
      2. (b) and (d)
      3. (a), (b), (c)
      4. All of the above
      5. None of the above

   ANSWER: 2
3. When applied to childhood neurodisability, the non-categorical approach dovetails well with the World Health Organization's International Classification of Function, Disability, and Health (ICF) because of ICF's emphasis on:
   (a) Describing individual functioning at various levels
   (b) Grouping of neurodevelopmental disorders on basis of underlying medical etiology
   (c) Understanding how contextual factors affect individual functioning
   (d) Prediction of health status on the basis of condition type
      1. (a) and (c)
      2. (b) and (d)
      3. (a), (b), (c)
      4. All of the above
      5. None of the above

   ANSWER: 1
4. Support for wider adoption of the non-categorical approach in the context of childhood neurodisability comes from:
   (a) Potential to leverage supports and policies aimed at a significantly sized population, even if individual conditions are uncommon or rare
   (b) Difficulty diagnosing neurodevelopmental conditions of childhood
   (c) Many treatments and interventions for children with neurodisability aim to ameliorate the *effects* of an underlying condition, rather than to reverse a clearly understood biomedical derangement
   (d) Research that shows greater functional heterogeneity *across* various chronic condition types than *within* conditions
      1. (a) and (c)
      2. (b) and (d)
      3. (a), (b), (c)
      4. All of the above
      5. None of the above

   ANSWER: 1

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Pain and Symptom Management in Neurodevelopmental Disorders: Sensory and Nociceptive Function/Pain and Symptom Management

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Learning Objectives

1. To learn about historical beliefs and scientific knowledge related to pain in children with intellectual and developmental disabilities (IDD).
2. To learn methods to assess pain in children with IDD.
3. To learn approaches to treating various types of pain in children with IDD.
4. To learn about current knowledge translation projects improving pain assessment and secondary caregiver education in IDD.
5. To learn about the challenges associated with studying and managing pain in IDD.

Highlights

- Assessment tools specifically designed for IDD include the Paediatric Pain Profile (PPP), the Non-communicating Children's Pain Checklist-Revised (NCCPC-R), and the Faces Legs Activity Cry Consolability Scale—Revised (r-FLACC). The Brief Pain Inventory (BPI) pain interference items are also recommended to assess pain interference with daily activities.
- Treatment plans should include (1) standardized pain assessment, (2) thorough and appropriate diagnostic testing, (3) treatment, (4) follow-up, and (5) communication.
- Available evidence supporting treatment approaches for hypertonia pain, procedural pain, postoperative pain, and pain and irritability of unknown origin (PIUO) was reviewed.

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Background and Significance

Pain is a functional response alerting us to danger and disease; yet, the adverse effects of poorly managed pain can be debilitating. Untreated pain that transitions from acute to chronic can have catastrophic comorbid consequences. Epidemiological estimates of chronic pain in IDD are exceedingly high ranging from 40 to 60% in clinical and large representative samples [1, 2]. In this chapter, we address issues specific to pain and symptom management for children with intellectual and developmental disabilities (IDD) associated with neurodevelopmental disorders. Despite evidence that children with IDD experience more painful medical conditions and procedures compared to their typically developing peers, critical unanswered questions remain about the nature of the pain experience in this clinical population [3–5]. There are likely several related

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and some unrelated reasons for a less than robust program of clinical research addressing pain in children with IDD. First, the more severe impairments associated with rare disorders (e.g., Rett syndrome and Prader-Willi syndrome) can be low-incidence conditions making it difficult to systematically address in a coordinated way participant screening, recruitment, and clinical care. Second, the associated or comorbid impairments including different combinations of intellectual/cognitive, motor, and communicative impairment make access to a complex subjective experience (i.e., "pain") difficult with the clinical corollary that it is tough to manage what you cannot measure. Third, part of the pathological pathway leading to the neurodevelopmental disorder may also affect sensory and nociceptive system neurodevelopment in ways that we do not understand scientifically and fail to appreciate clinically. Finally, there may well be (are likely) socio-cultural variables operating including beliefs that individuals—children or adults—living with IDD do not feel pain or have a heightened threshold for pain [6–8]. This view, although not well substantiated empirically, leads people to believe that children or older individuals, as well, with IDD who experience noxious stimuli would have reduced pain sensation compared to their typically developing peers having the same noxious experience. Emerging evidence suggests the contrary [9, 10].

In this chapter, we will expand on each of these salient topic areas that impact the fledging field of clinical pain research specific to IDD. We will explore the epidemiology of pain in IDD, the evidence supporting claims of heighted pain threshold in IDD, approaches to pain assessment and treatment, and the need to move from assessing the presence of pain to assessing the underlying mechanisms of pain. Finally, we will discuss the extent to which the current research evidence has influenced clinical practice (i.e., knowledge transfer) and summarizes the continuing issues while highlighting future avenues which will move the field of pain in IDD forward.

Approaches and Challenges to Pain Assessment in IDD

Sensory Measurement

Separate from historical socio-cultural reasons or perhaps because of them, a general theory or perspective of a heightened pain or sensory threshold specific to individuals living with IDD may have come about because of anecdotal and clinical observations consistent with apparent dampened pain responses. This theory of apparent reduced sensitivity may have been perpetuated, in part, due to the inability to self-report pain because of associated motor or communicative impairments, particularly for individuals with more significant impairment and disability [11]. It is certainly the reality that caregivers and health-care professionals often report pain signs as ambiguous, paradoxical, altered, blunted, or confused with stress or arousal [12]. Indeed, there is great heterogeneity within the population of individuals with IDD and some individuals may experience altered pain sensation. However, there is no compelling explanation for why all individuals with IDD would have such globally affected physiological systems such that the universal experience of pain is reduced for this vulnerable group [13].

Given the above preamble, there is controversy in the literature regarding the concept of pain threshold and sensation in individuals with IDD. On one hand, there is some research aligned with the position that at least a proportion of individuals with IDD have a heightened pain threshold resulting in no pain response [8]; while on the other hand, other research clearly shows individuals with IDD do react to pain in a typical or even hypersensitive manner [14, 15]. Part of the problem is the approach and methods used to address the issue scientifically. For example, early reports in this field by Biersdorff et al. [6] relied on proxy-report and peoples' opinions of whether they think individuals with IDD experience pain the same as individuals not so labeled, whereas other approaches rely on more direct measurement. The disagreement in the literature creates confusion for health-care professionals and caregivers and may have interfered with progress toward better clinical practice.

A search for peer-reviewed articles specific to establishing and estimating sensory and pain thresholds of individuals with IDD yields only 17 studies ([8, 9, 14, 16–29]; see Table 43.1). Four studies used a survey method and two were case studies or case series. Eleven studies used an empirical design and nine of those eleven studies included a control group. Although there was variability in study design, the strength of the evidence, and study quality, there are several important "take aways." First, several of the studies reviewed did conclude that individuals with IDD have elevated pain or sensory thresholds (meaning the individuals with IDD were less sensitive to pain); however, these studies were all based on the anecdotal report of parents, caregivers, or health-care professionals. Second, there were six studies that fell short of testing pain thresholds but did apply sensory stimuli and measured the behavioral reactivity of the participants with IDD. All six studies found that individuals with IDD were reactive to various tactile sensory stimuli and the majority of studies concluded that those with IDD were more (not less) reactive compared to controls. Third, of the five studies that conducted pain threshold testing in IDD compared to control groups all five studies concluded that individuals with IDD had normal or even lower pain thresholds. These studies, therefore, concluded that individuals with IDD were equally sensitive or more sensitive to pain compared to their typi-

Table 43.1 Evidence examining pain thresholds in individuals with intellectual and developmental disabilities (IDD)

Study design	References	Sample	E	C	Claim
Relied on anecdotal report of parent, caregiver, or health-care professional regarding pain sensitivity	Downs, et al., (2010; [16])	*n* = 646 parents (RS)	✗	✗	• 65% reported decreased pain sensitivity on survey
	Biersdorff, (1994; [8])	*n* = 123 parents (IDD)	✗	✗	• 25% reported heightened thresholds
	Butler et al., (2002; [17])	*n* = 66 caregivers (PWS)	✗	✗	• Hypothesized heighted pain threshold to account for failed detection of illnesses
	Couston et al., (1954; [18])	*n* = 7 case series IDD	✗	✗	• Reported absence of pain expression when presenting with assumed painful medical conditions
	Devarakonda, et al., (2009; [20])	*n* = 1 case study (RS)	✗	✗	• Case of reduced need for postoperative analgesia, suspected heightened pain threshold
	Kankkunen et al. (2010; [21])	*n* = 181 nurses (IDD)	✗	✗	• Nurses considered pain threshold elevated in non-verbal IDD
Sensory testing procedures determined how individuals with IDD respond to stimuli	Hennequin et al., (2000; [22])	*n* = 26 children and adults with DS	✓	✓	• Sensitive to pain • Slower to respond • Less precise localization
	Stengel, et al., (1955; [23])	*n* = 107 individuals with IDD	✓	✗	• Sensitive to pain • Response time to other diagnoses
	Symons et al., (2010; [24])	*n* = 44 adults with IDD (*n* = 29 had self-injurious behavior [SIB])	✓	✗	• Increased reactivity to active vs. sham stimulus trials • Females more reactive • Individuals with SIB more reactive
	de Knegt et al. (2015; [25])	*n* = 188 adults with DS	✓	✓	• Tactile responsiveness • DS with lower intelligence less able to discriminate sharp/dull
	Benromano et al. (2017a; [19])	*n* = 18 adults with CP (72% with ID)	✓	✓	• CP with ID were more reactive (self-report, facial expression) to noxious pressure compared to CP without ID and controls
	Benromano et al. (2017b; [9])	*n* = 16 adults with ID (50% unspecified ID; 50% with DS)	✓	✓	• Adults with ID had more facial reactivity and greater pain evoked potentials compared to controls
QST procedures lead to warranted conclusions regarding pain thresholds	Weidenbacker et al. (1963; [26])	*n* = 30 children with CP	✓	✓	• **TH** pressure pain for non-ambulatory CP compared to ambulatory CP and controls
	Defrin, et al., (2004; [14])	*n* = 25 with ID (56% unspecified ID; 44% DS)	✓	✓	• **TH** noxious stimuli
	Priano et al., (2009; [27])	*n* = 14 PWS	✓	✓	• **TH** heat pain • **TH** cold pain
	Riquelme & Montoya (2010; [28])	*n* = 29 children and adults with CP	✓	✓	• **TH** tactile non-noxious stimuli • **TH** noxious stimuli • Enhanced SEP amplitudes
	Valkenburg et al. (2015; [29])	*n* = 41 children with DS	✓	✓	• **TH** heat pain

E empiric study design, *C* control group included, *X* No; ✓ = Yes, *CP* cerebral palsy, *DS* down syndrome, *RS* Rett syndrome, *PWS* Prader-Willi syndrome, *SIB* self-injurious behavior, *ID* intellectual disability, *TH* threshold, *SEP* sensory evoked potential

cally developing peers. Considering the evidence to date, there does not appear to be a scientifically compelling reason to conclude or assume that all individuals with IDD or with specific diagnosis have uniformly heightened pain thresholds or that they do not experience pain regardless of the situation. If fact, in the more rigorously conducted studies, it seems the opposite.

Observational Measurement

As alluded to, the current "gold standard" for assessing the presence of pain remains verbal self-report. This is difficult if not impossible for individuals who cannot reliably self-report or who do not have formal communication systems to report their experiences. As such, there remains a need to develop alternative assessment methods. One possible approach is the use of direct observation for behavior expression consistent with pain signs. In this section, we describe the development of a fine-grained observational-based coding system for research purposes and by doing so illustrate and highlight a number of decision steps toward developing a useful measurement protocol that are not often described or discussed in scientific manuscripts.

We (CB, AM, FS) began by modifying the Pain and Discomfort Scale (PADS; [30]), a set of pain/discomfort-

related vocal (e.g., yell), facial (e.g., furrowed brow), motor (e.g., pulling arm away from stimulus), and physiological (e.g., gasp) behaviors derived from the Non-Communication Child's Pain Checklist [31]. The PADS system was designed as a pain/discomfort clinical screening tool for non-verbal individuals with severe IDD. Versions of this coding system have been used across protocols, diagnoses, and ages, including adults with intellectual disabilities [32], Rett syndrome [33], neuronal ceroid lipofuscinosis [34], and typically developing preschoolers [10].

We implemented a program of research in which we have adapted the PADS, designed as a screening measure, to be used as a research-based observational coding tool, particularly when "paired" with an examination procedure. Here we describe our approach and its issues over the course of a program of pain research specific to girls and women with Rett syndrome, a rare genetic neurodevelopmental syndrome with severe communication impairment in most individuals, and potentially abnormal or atypical somatosensory/nociceptive function [35]. The context of measurement occurs during a modified quantitative sensory test (mQST). The mQST consists of seven stimuli: sham (no stimulus applied), light touch von Frey, pin prick, cool, deep pressure, repeated von Frey to test temporal summation, and heat. All stimuli are applied to the back of the calves. The von Frey monofilaments are small handheld QST tools that provide calibrated tactile input to the skin – used for light touch (2 g force) as well as repeated touch (60 g force). Our purpose in using a QST approach is to "interrogate" in a global and non-invasive way the functional integrity of the somatosensory axis. Because all participants in our Rett syndrome clinical research protocols cannot self-report using the spoken word, we have been adopting and adapting clinical research protocol procedures that rely on behavior observation during sensory testing. Given there is no language (e.g., "I can feel that"), we time-lock observations to stimulus application and then look for codable (i.e., operationally defined) behaviors.

In the first iteration, coders individually scored each of the 15 behaviors from the PADS on a 0–2 scale for each application of each stimulus (26 applications total). Coders began by scoring all 15 behaviors for the right-hand application of light touch, the same 15 behaviors again for the left-hand application, then for the application on each foot. They then repeated the process for the remaining 6 stimuli, totaling 22 applications of mQST stimuli. This method led to 390 items to score for each participant (Fig. 43.1). Scores within each stimulus were summed for a stimulus reactivity score. While this system provided a fine-grained analysis of pain/discomfort reactivity to each stimulus, it was impractical for long-term use. Because of the high level of required inter-rater reliability for trainees (at least three videos above 90% agreement of scored behaviors with the lead coder), we found inability to consistently train coders to the reliability standard, even over a 12-month training period. Additionally, the coding burden of each video was extremely high, taking over three hours to code one five-minute mQST video. These resource demands were not feasible, thus we tried to decrease coder burden while still maintaining the full set of pain/discomfort-related behaviors.

In the second iteration, we used the same 15 behavioral definitions as the first system, but grouped them into five behavior class codes: vocal, upper face, lower face, body, and physiological. Each category contained two to three of the original individual behaviors. These behavior classes were then scored on a 0–3 scale. For example, one tongue thrust and one mouth open would result in a score of 2 for the lower face behavior class, instead of scoring them individually as a 1 in the previous system. Further, coders scored all applications of each stimulus as one application segment instead of scoring each application individually. Totals of the behavior classes for each stimulus became the stimulus reactivity score. These groupings resulted in only 35 items to score (Fig. 43.1). This system resulted in trainees taking four to six months to reach the 90% inter-rater reliability goal, on average, with all trainees successfully completing training. In addition, this system reduced coding time from three to one and a half hours per mQST video.

When comparing both systems' reactivity scores, we found a similar pattern across stimuli (Fig. 43.2) and total scores (Fig. 43.3; $\rho = 0.75$, $p < 0.0001$). Because of the drastically reduced training and coding burden with the second behavior class scoring system, we have continued using the second coding system in future work. We included this detailed description of an observational protocol development because rarely can one find the steps and issues addressed along the way that research groups take to get to a usable and useful measurement tool. While the issues may not be unique to our measurement context, they do underscore the multiple issues that need to be considered when adopting and adapting a direct observational measurement approach for pain behavior for high-quality clinical research in specialized samples from vulnerable populations including IDD.

Proxy Report

Although many children with IDD may have the capacities to provide basic forms of self-report, self-report may be best used in conjunction with other methods of assessing pain [36]. Researchers have found that children and adults with mild IDD who have verbal skills were able to assess pain in others accurately; however, when experiencing pain themselves they had difficulty reporting their own pain with similar accuracy [37, 38]. This may be because being in pain can reduce cognitive capacity [4]. Further, some individuals with IDD, although they may have strong verbal skills, may lack the ability to understand order and magnitude (e.g., 4 is twice as great as 2) of a cognitive skill that typical self-report scales necessitate.

	Stimulus 1				Stimulus 2				Stimulus 3			
Video Times												
Limb	Right Hand	Right Hand	Left Hand	Left Foot	Right Hand	Right Hand	Left Hand	Left Foot	Right Hand	Right Hand	Left Hand	Left Foot
Vocalizations Whimper, cry, moan, "ow"	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA
Upper Face Brow raise, furrowed brow, change in eyes	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA
Lower Face Change in mouth, lip pucker, clench/grind teeth/tongue thrust/bites	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA
Body Movements Move away/guard, flinch	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA
Physiological Signs Tears, gasp, breath holding, noisy breathing	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA	0 1 2 NA
Total Score												

	Stimulus 1	Stimulus 2	Stimulus 3
Video Times			
Vocalizations Whimper, cry, moan, "ow"	0 1 2 3 NA	0 1 2 3 NA	0 1 2 3 NA
Upper Face Brow raise, furrowed brow, change in eyes	0 1 2 3 NA	0 1 2 3 NA	0 1 2 3 NA
Lower Face Change in mouth, lip pucker, clench/grind teeth/tongue thrust/bites	0 1 2 3 NA	0 1 2 3 NA	0 1 2 3 NA
Body Movements Move away/guard, flinch	0 1 2 3 NA	0 1 2 3 NA	0 1 2 3 NA
Physiological Signs Tears, gasp, breath holding, noisy breathing	0 1 2 3 NA	0 1 2 3 NA	0 1 2 3 NA
Total Score by Stimulus			

Fig. 43.1 Comparison of score sheet for three of six stimuli for the individual behavior method (left) and the behavior class method (right)

Most often proxy report is used to assess pain for individuals with IDD. Proxy reports are frequently obtained from a parent or caregiver who knows the child best. These reports may be ascertained in the form of a descriptive verbal report, a numeric rating scale, or a pain observation tool where the parent rates their child's pain behaviors such as vocal or facial expressions. Proxy report of pain in children with IDD relies on the proxy's ability to detect and recall the severity of pain signs such as crying, moaning, limb and body movements, postures, and facial behaviors [31]. Obtaining a proxy report of pain is sometimes the only feasible option and this approach is certainly superior to not assessing pain at all. It is important, however, to understand that the accurate assessment of another individual's internal

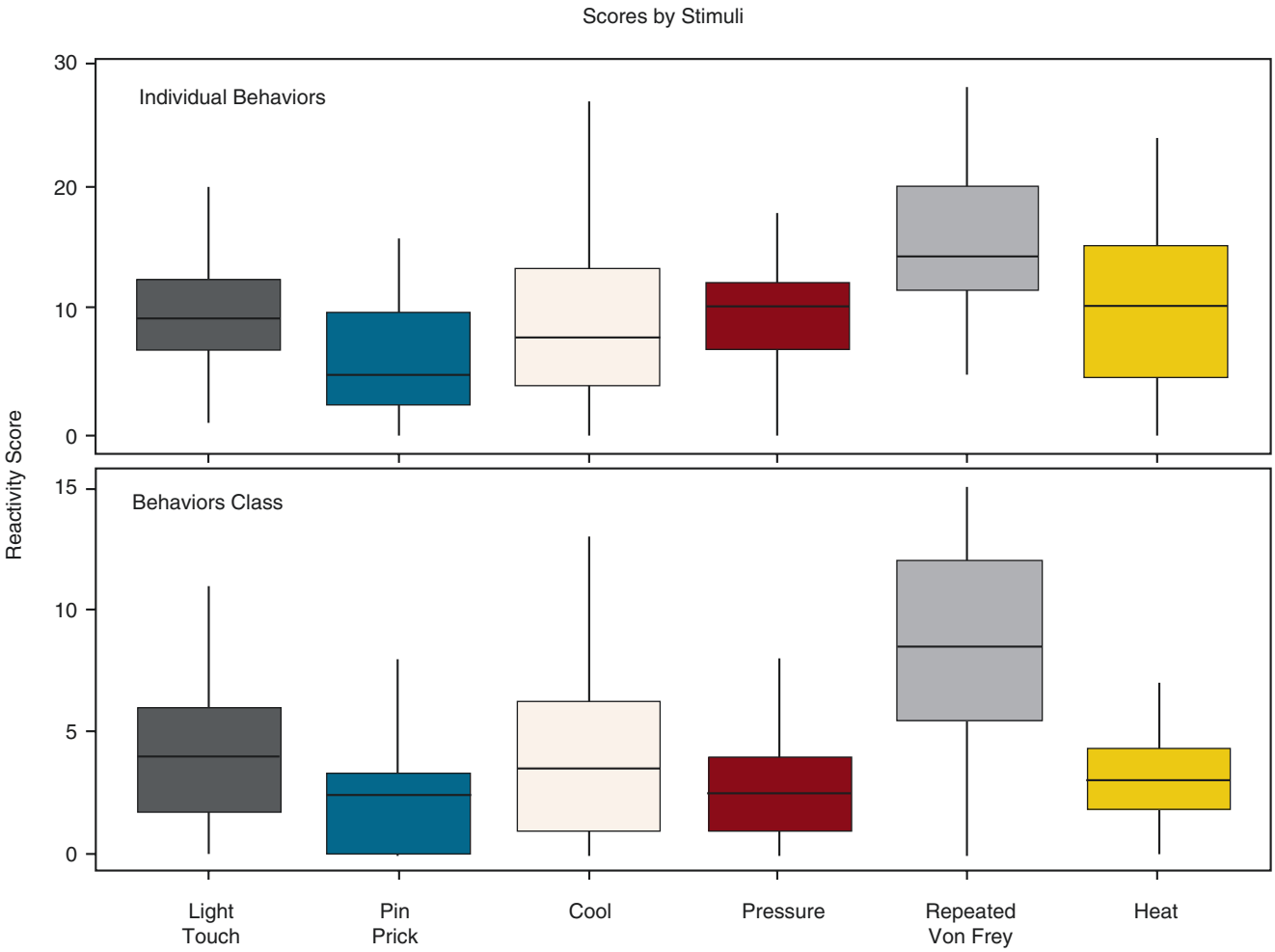

Fig. 43.2 Comparison of reactivity scores by stimulus for the first, individual behavior scoring method (top) and the behavior class scoring system (bottom)

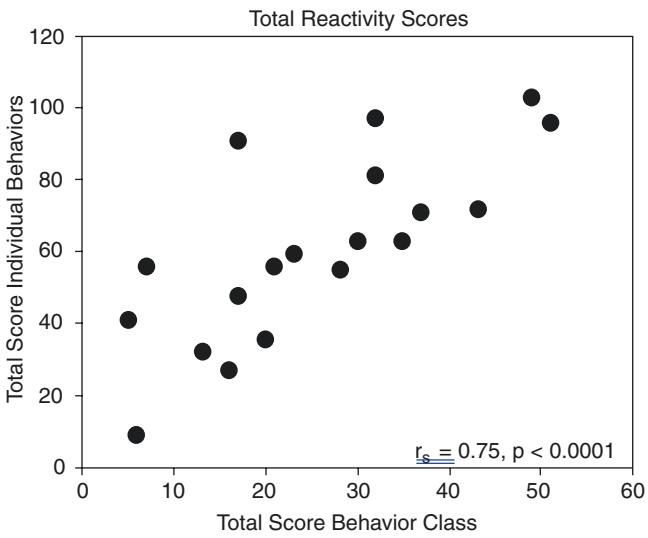

Fig. 43.3 Total scores from the two coding systems

physiological and psychological state is not without serious challenge. In fact, assessing another's pain experience has been compared to a "mind reading" task [36]. Obviously, this approach requires skill, sensitivity, and astute judgment and even then, at least for some, there is no way to determine if the proxy assessment is accurate.

Parents of children with IDD can have difficulty identifying pain in their children. In one study, parent proxy reports were compared with objective pain observations. It was found that parents were more accurate at assessing pain in typically developing children compared to assessing pain in their children with autism [39]. Similarly, another study found that parents were generally inaccurate at identifying their child's dental pain unless obvious physical signs (i.e., swelling, redness) were present [40]. The lack of identification resulted in children with IDD waiting on average 3.7 months longer to see a dentist. These studies suggest that parent proxy ratings may not be accurate; however, more

research is needed to clarify the circumstances in which this is more or less of an issue (e.g., home vs. clinic, type of pain, parent beliefs about pain).

There is little known about what factors influence the proxy's pain ratings. Factors such as the parent's physical and mental health, personality, coping, optimism, and self-esteem likely influence parent ratings although most of these factors have not been systematically studied [41]. For example, in studies on proxy ratings of quality of life in children with CP, quality of life scores were equally or more influenced by parental psychosocial distress than the child's severity of impairment [42, 43]. White-Koning et al. [44] found that 64% of the time parent proxy report and the child with cerebral palsy's report of quality of life did not match. The parent's high levels of stress or the child's high levels of pain were the key predictors of discordant ratings. These studies, although not all specific to pain, give initial insight into how parental psychosocial distress may factor into proxy pain ratings.

Parents also face challenges in learning to understand and detect their child's pain. As noted earlier, children with I/DD may show ambiguous or paradoxical signs of pain that could easily be confused with non-pain related distress [45]. Carter, McArthur, and Cunliffe [46] conducted a qualitative study exploring how parents assess pain in their children with I/DD. The researchers found that parents reported assessing pain to be a complex process that required them to draw on skills and knowledge about their child that they had gained over many years. Learning to be attentive to these signs may not come naturally and other factors may impede a parent's ability to learn these subtle signs. A study of mothers with young infants demonstrated that mothers differ in their capacity to detect changes in their infant's cry and the mother's ability to learn their infant's cry patterns was affected by marital happiness, work/home conflict, and depression [47]. This study suggests that the ability of parents to effectively learn their child's unique pain signs may be determined by the parent's personal learning capability and impeded by similar psychosocial factors already described above. This is troubling given that (1) adults who maintain a care giving role are known to have significantly higher rates of depression and distress compared to non-caregivers [48] and, 2) parent proxy reports are typically the sole determinant of a child with IDD's pain severity.

Biomarkers

Use of pain-related biomarkers may offer novel insights into biological activity associated with nociceptive/stress and inflammatory processes. A biomarker approach to assessing pain-related experiences may be especially relevant among individuals with IDD, where communication of pain and distress may be altered or diminished. A few reports have been published related to biomarkers in IDD. Symons et al. [49] found that the majority of salivary metabolite, neuropeptide, cytokine, and hormone levels were higher in children with CP who had pain (based on parent report) compared to those who did not have pain. Salivary analytes did not differentiate between CP severity, gender, or prematurity status. Oberlander et al. [50] assessed measures of autonomic cardiac modulation (heart rate variability, high frequency power, etc.) during blood collection by heal lance in a group of very low birth weight infants with parenchymal brain injury. The majority of these infants were later diagnosed with CP. Compared to controls, the infants with brain injury did not differ in physiological biomarkers of pain reactivity during heel lance.

In spite of progress to understand biomarkers in the context of pain in IDD, many IDDs are not unitary diseases but are behaviorally defined umbrella disorders (e.g., CP, autism) leading to considerable variability in physiological and behavioral phenotypes. Second, pain does not have a specific biological marker [51]. Third, we may not know whether we are measuring something specific to the IDD diagnosis, chronic pain, or both. Because pain is a complex construct consisting of sensation, perception, and expression influenced by psychosocial processes, it seems reasonable and important to assess across all these domains, including biomarkers consistent with activated nociceptive/stress and inflammatory pathways. This is stated with the caveat that it is unlikely biomarkers alone will readily support the inference that "the individual is in pain."

Neuroimaging

Recent advances in neuroimaging, such as the application of electroencephalography (EEG), magnetoencephalography (MEG), structural and functional magnetic resonance imaging (MRI/fMRI), provide critical insights into the human brain structures or function related to the pain experience. Each of these neuroimaging techniques offers different temporal, spatial, or spectral resolutions, but all carry the potential to objectively characterize a neuronal "signature" for pain perception in individuals with IDD. An fMRI study in adults with Alzheimer disease (AD) has tested whether the speculation by which AD reduces central brain processing of painful stimulations can be supported by neuroimaging evidence [52]. When the brain functional response to painful stimulation was compared between adults with AD and controls, there was no evidence for reduced central function; levels of activation of pain-related brain regions such as the anterior midcingulate cortex and the insula were similar between the groups [52]. On the contrary to the speculation, AD patients showed increased activation in the dorsolateral prefrontal cortex (DLPFC), suggesting an increased cognitive perception of

painful stimulation. These findings might have important clinical implications, which raise concerns about pain management protocols currently used in this population. Using fMRI, it was shown that adults with DS have reduced volume and connectivity of the orbitofrontal cortex (thought to be involved in pain modulation) [53] which might explain the higher sensitivity to pain observed in DS [29]. High-functioning adults with autism have also shown higher sensitivity to pain stimulation, reflected by increased anterior cingulate cortex activation, but only during pain anticipation, and not during pain perception [54]. These results might reflect altered attention to anticipatory cues of upcoming painful stimuli. Other neuroimaging studies in adults with autism have examined the neural correlates of empathy (i.e., perceiving others in pain), which is a key deficit in autism [55–57]. When young adults with autism presented with complex stimuli of social pain, subjects with autism exhibited a reduced activation of the anterior insula which correlated with the severity of autistic symptoms. Interestingly, compared to controls, autistic adults did not show coupling between their self-report of the intensity of their social pain experience and functional connectivity of the insula; however, they did show increased activation of the hippocampus. The authors interpreted this observation as a possible compensatory mechanism of using activation of a region involved in memory processes instead of using activation of regions involved in accessing embodied signal (AIC). However, evidences related to brain function in the context of more simple stimuli depicting vicarious physical pain or painful expressions are inconsistent. While one study showed a decreased activation of the AIC [56], others have shown no differences in the brain response compared to healthy controls [55, 57].

In children, neuroimaging tools have also been used to study the neural correlates of self-injurious behaviors which are common in autism, and can result in visible injuries [58, 59]. First, parents provided information on the self-injurious behaviors using a standardized questionnaire to divide the autistic group to high and low levels of self-injurious behavior. Children with ASD aged 7–15, and aged matched typically developing controls were scanned using both MRI and diffusion tensor imaging. Healthy controls did not differ from children with ASD; however, within the group with ASD, high levels of self-injurious behavior were associated with reduced microstructure of the internal capsule and decreased cortical thickness of the right superior parietal lobe and the lateral somatosensory cortex. These differences may suggest altered somatosensory capacity for those with ASD and self-injurious behavior. Altered somatosensory response was also reported in an MEG study in 7–12-year-old children with ASD, reflected by lower amplitude of the early evoked response in somatosensory cortex compared to healthy controls [60].

Collectively, these results suggest that neuroimaging can provide insights into neural correlates of the pain experience in IDD. However, most of the available neuroimaging techniques require complex instrumentation which is very costly and not easily accessible for clinical procedures in the day-to-day routines. Nonetheless, these methods are valuable in understanding neural plasticity in relation to pain perception both in adults and in children with IDD.

Current Assessment and Treatment Approaches

Assessment

The difficulty of pain assessment is amplified in the presence of cognitive, communicative, or motor impairments associated with IDD. As such, the verbal and cognitive functioning relied on for self-report of pain is often compromised. Part of the challenge is also in the varied nature of IDD. No one assessment approach is appropriate for all individuals with IDD or even all individuals with a given diagnosis, because children have various levels of cognitive functioning, verbal abilities, and motor impairments. Additionally, multiple people are often involved in the care of children with IDD (e.g., parents, teachers, physicians, nurses, physical therapists) and pain assessment and management could often be regarded as someone else's responsibility.

When self-report is unavailable or unreliable, determining the source of the pain can be a challenge. Understanding common sources of pain in IDD may aid providers' ability to effectively detect pain and provide appropriate treatment. Musculoskeletal pain is frequent and can present as hip dislocation/subluxation, spasticity, dystonia, and musculoskeletal deformity [61] but it is common to detect multiple sources of pain (e.g., gastrointestinal, accidental, and seizure related pain; [62]). For an extensive review of pain sources in CP, see Vogtle et al. [63].

The most important choice particularly from a clinical perspective may not be which scale to use but rather to make a decision and commitment that pain will be assessed systematically and be aware that there are multiple measurement tools to choose from. Appropriate measurement tools are now relatively straightforward to access in an evidence-based Chronic Pain Assessment Toolbox for Children with Disabilities (available at www.hollandbloorview.ca/toolbox; [64]). Worth noting are those tools that were designed or refined specifically for children with IDD, which include the Pediatric Pain Profile (PPP; [65]), the Non-Communicating Children's Pain Checklist-Revised (NCCPC-R; [31]), and the Faces Legs Activity Cry Consolability Scale – Revised (r-FLACC; [66]). The PPP provides the option to complete an in-depth individualized pain profile for each child as well as a brief (20-item) pain assessment which is more practical for clinical and repeated use. The NCCPC-R is a 30-item

assessment that can be completed after a 5–10 min observation of the child. Both the PPP and NCCPC-R provide cut scores to help interpret the pain intensity level and interpret the need to provide treatment. The r-FLACC has five items that are each scored from 0 to 2, resulting in a traditional 0–10 pain score. The r-FLACC can be individualized to account for idiosyncratic pain behaviors and is most suited for inpatient care and acute pain events.

There is emerging evidence supporting the use of the Brief Pain Inventory (BPI) pain interference subscale for documenting pain's impact on activities of daily living, such as sleep, mood, and self-care [67, 68]. The 12 pain interference items of the BPI have shown good to excellent psychometric properties as a self-report tool for adults and youth with IDD without cognitive impairments [69–71] and as a proxy-report tool for parents of children with CP [67]. This is the first assessment tool that appears to be applicable across a range of cognitive/mobility levels and as a self- and proxy-report tool. The IMMPACT guidelines for pain assessment support the use of the BPI pain interference items to better characterize the multifaceted nature of pain and pain's impact on function rather than relying on pain intensity scores alone [72, 73].

Treatment

To date, the majority of research evidence has focused on documentation of pain experience and burden in IDD as well as the creation of multiple pain assessment tools and IDD-specific evidence to guide treatment remains limited by the lack of empiric evidence-based approaches.

It is important to create a plan for pain management, especially in complex cases. The plan should include (1) ongoing pain assessment, (2) diagnosis, (3) treatment approaches, (4) regular follow-up, and (5) ensuring effective communication among the individual, the family, and all health-care professionals. First, a thorough and appropriate pain assessment should be completed on a regular basis. At minimum, a pain assessment should be completed at each provider visit. Ideally, parents/caregivers will be completing pain assessments daily at home and/or at school. Routine assessment of pain will provide important information to determine if pain management approaches are beneficial. Second, a pain diagnosis should be sought. Diagnosis will require the identification of the underlying pathology causing pain. Careful evaluation is required, including: comprehensive evaluation of all systems, diagnostic testing, clinical judgment, information from parents/caregivers, pain exacerbating and mediating factors, empiric medication trials, and careful follow-up [74]. However, even with all diagnostic avenues explored, a diagnosis may not always be possible and a treatment approach must still be developed. Third, treatment approaches (more fully described below) should be systematic and guided by the pain diagnosis as well as the information gathered in step 2 [74]. The physiological mechanisms of different pain types (nociceptive, inflammatory, neuropathic, etc.) should be considered when treating pain in children with IDD [75]. Consider the WHO guidelines: by mouth, by the clock, and by the ladder (WHO, 1996, p. 36). For the perioperative setting, consider the American Society of Anesthesiologists published practice guidelines for acute pain management, with special recommendations for pediatric and cognitive impaired patients (2012). Fourth, close follow-up is important. Ongoing pain assessments can detect the degree to which a pain management strategy is helpful and determine whether changes to the treatment approach are warranted. Finally, work is needed to establish coordinated communication and decision-making between the individual, caregivers, and all providers involved.

Given the paucity of evidence on treatment approaches for pain in IDD, we will report on a summary of pain treatment approaches in CP because it is available and CP is the largest single developmental disability condition leading to pediatric physical/motor disability. A recent CP-specific systematic review found level II evidence for non-pharmacological interventions for procedural pain, pharmacological approaches to postoperative pain, and the use of intrathecal baclofen for chronic pain associated with hypertonia. This review and other studies will be reviewed below.

Hypertonia pain. Various treatment approaches focus on management of hypertonia, including Botulinum toxin injections, gabapentin, and oral and intrathecal baclofen. However, the research evidence supporting these particular treatment approaches is non-existent, sparse, or inconclusive for most approaches. Studies assessing the utility of botulinum toxin injections are mixed, with observational studies documenting improvement but a well-designed randomized controlled trial comparing botulinum to saline showed no difference in pain outcomes [76]. There are a handful of studies assessing intrathecal baclofen in which pain was explicitly measured. In three studies, pain was prospectively and explicitly assessed in ITB trials specific to children with CP [77–79]. In all three studies, improvements in pain/comfort dimensions were documented in the ITB group compared to controls. McCormick et al. compared pain scores for patients randomized to ITB compared to oral baclofen and found no difference in pain outcome; however, the sample was not specific to CP ($n = 10$ CP patients out of 62; [80]). In another study, gabapentin for hypertonia was evaluated, with 33 of 84 participants having CP. Improvements in pain were only seen when gabapentin was used in combination with ITB or deep brain stimulation, not when gabapentin was used alone [81].

Procedural pain. Children with IDD frequently undergo painful procedures for diagnosis or treatment. In one large multicenter study conducted in Italy, it was found that despite

children with IDD receiving more support during needle pain procedures they demonstrated higher levels of pain and anxiety compared to controls [82]. Children with CP often undergo intramuscular injections of botulinum toxin injections (BTI) to treat hypertonia. Pain associated with the procedure is typically managed with inhaled nitrous oxide/oxygen and/or anesthetic cream. This combination kept pain below the therapeutic threshold for approximately half of participants [83, 84]. When patients are not able to tolerate mask breathing or have negative reactions to nitrous, rectally administered midazolam and racemic ketamine appears to be a viable alternative [85]. Additionally, when conscious sedation approaches are ineffective, general anesthetic can be used [86]; however, the long-term effects of this approach are not well studied.

For pain associated with venipuncture, there was a level II evidence to support cooling vibration (i.e., a device called a Buzzy). The one randomized controlled trial found that nonverbal children with cognitive impairments (CP, epileptic encephalopathy and genetic syndromes) had pain scores on the NCCPC-PV below the cut score for pain 91% of the time when cooling vibration was used (compared to 61% for controls; [87]). A systematic review in typically developing children supports the utility of the Buzzy device for reducing needle pain [88]. Venipuncture pain in typically developing children has been widely studied. Evidence suggests it is important to use (as appropriate) topical anesthetics (including J-tip needles), positioning the child for comfort, and age-appropriate distraction [89]. Evidence in typically developing children is emerging to support more effective and sustained distraction using hand-held computers [90], distraction kits [91], and virtual reality [92], with one case study reporting reduced pain for an adolescent with CP using virtual reality during postsurgical physical therapy [93].

Especially when children with IDD are repeatedly exposed to painful procedures for treatment of physical disabilities, it is important for providers to recognize and manage fear, anxiety, and pain. Further research and education is required to support physician and nursing skills in recognizing, measuring, and managing pain and anxiety in children with IDD [82]. Both pharmacological and nonpharmacological approaches to procedural pain management should be explored [89].

Postoperative pain. Providing adequate analgesia in the postoperative period is a challenge in children, made especially challenging in children with IDD. This is due, at least in part, to concerns about the risk of side effects and toxicity as well as the complete lack of evidence to inform proper analgesic dosing in these children. Observational studies suggest the tendency is to provide sub-therapeutic doses of analgesics to children with severe IDD [94, 95]. However, less than 10% of surveyed anesthetists report giving lower doses of intraoperative opioids in children with IDD [96]. Postoperative pain management specific to lower limb surgery in CP included epidural analgesia with bupivacaine [97], continuous infusion magnesium sulphate [98], and preoperative popliteal block with general anesthesia [99]. These studies provided strong quality level II evidence for these approaches. Following selective dorsal rhizotomy in children with spastic diplegia, there was evidence of adequate pain management using morphine with intrathecal and epidural administration [72].

Pain and irritability of unknown origin (PIUO). In children with severe neurological impairments, who are typically non-verbal, non-mobile, and have limited cognitive abilities, there can be a problem of ongoing, unexplained, and difficult-to-treat pain and irritability. Children with severe neurological impairments have marked differences in their nervous system because of differences in brain anatomy, injury to the developing brain, disruptions in healthy cell metabolism, or a host of other disruptions. Thus, the same assumptions about pain signals and responses cannot be made. Recently, a pathway has been published to support more uniform treatment for PIUO [74] and testing of this pathway is underway. The pathway involves multiple steps including initially obtaining a history and physical exam, conducting studies to determine possible causes (e.g., blood tests, X-rays, and dental exam), and consideration of an empirical drug trial. If these steps result in no identified pain source or improvement in symptoms, then treatment should include further testing, a trial of gabapentin, non-pharmacologic interventions, management of pain triggers (e.g., constipation), and use of medications for breakthrough symptoms. For further information, see Hauer & Houtrow [74].

Research evidence available to guide clinical approaches to pain management in IDD is generally of low quality and weak design [72]. As a result, pain treatment approaches are often trial and error as there is not enough evidence from trials to state definitively which approaches will be most successful for individual patients. It is noteworthy that no studies examined treatment targeted to central pain excitatory mechanisms such as central sensitization. Further work is also needed to identify the value of non-pharmacological and multidisciplinary approaches to treat chronic pain in IDD—as these strategies are widely adopted in chronic pain practices in non-IDD patients.

Knowledge Transfer

Despite decades of documentation, there is little to no evidence suggesting that chronic pain as an outcome and secondary condition in IDD is routinely assessed let alone treated consistently or effectively (most commonly treatment is empiric—trial &error) [100]. Recently, however, steps have been taken to improve pain assessment practices in ambula-

tory clinics and to educate caregivers of individuals with IDD on pain assessment and treatment approaches. The Holland Bloorview Pain Assessment Toolbox for Children with Disabilities was created to provide a practical guide to implementing thorough, standardized, and appropriate pain assessments into clinical practice [64, 101]. The toolbox includes 15 tools that are fully described in the toolbox and vetted by clinicians who are experts in the care of children with disabilities. Tools include 7 assessment tools appropriate for assessing chronic pain interference and 8 tools to assess chronic pain coping. As of the writing of this chapter, the toolbox webpage has been visited over 8000 times and the toolbox has been downloaded over 140 times (Townley, personal communication). A multicomponent knowledge translation strategy facilitated implementation of the toolbox at the initial site in 2014. This led to adoption of standardized pain assessment practices in seven ambulatory clinics. Significant changes in pain screening and assessment practices occurred, with the percentage of patients having a completed pain assessment increasing from <2% pre-Toolbox to 53% post-Toolbox implementation. Undertaking implementation of the toolbox adoption required the extensive support of a non-clinical knowledge translation team [101].

Secondary Caregivers—A Literature Gap, Current Work, and Continued Future Directions

Despite the important role that caregivers of people with IDD often play in the pain assessment and management process, caregiver-based research has largely focused on parents and health-care professionals. While there is certainly still work to be done in this area, it is important to also include secondary caregivers (e.g., respite workers, teachers) in research. Indeed, these caregivers are unique in that parents may not always be available to interpret behavior/determine a course of action, and parents have underscored the need for professionals working with their children to be skilled and knowledgeable with respect to pain [102, 103].

Initial work exploring respite workers' beliefs and knowledge regarding pain indicated that pain and disability-related beliefs held by caregivers may impact, and in some cases predict, care decisions [104]. This may be problematic given that some beliefs may be inaccurate. Consistent with this assertion, a research group in United Kingdom found that of 33 residential services for adults with I/DD audited, none met a set of researcher developed standards related to pain assessment and management [105]. These standards included basic knowledge points that were also lacking in Genik et al. [104] such as inaccurate beliefs about the pain threshold of people with I/DD. Deficits in pain-related knowledge, skills, confidence, appropriate resources, and need for related education have been highlighted in preliminary literature in Canada, the United Kingdom, and the United States (e.g., [104–106]).

At present, some work has begun to explore the impact of pain-related training in respite, school, and residential settings with positive findings such as increased pain-related knowledge, changes in beliefs, and intent to use knowledge and strategies learned [107–109]. For example, Genik et al. [107] have developed the empirically informed *Let's Talk About Pain* program designed to educate respite workers about pain, pain assessment, and pain management in children's respite settings. Initial results from a pilot study suggested the training program resulted in a) immediate increases in pain-related knowledge, b) improved staff confidence and skill in assessing and managing pain, and c) positive endorsements of the training. A randomized controlled trial of this program is currently underway which will explore not only the impact on knowledge, perceptions, and impressions immediately following the program, but also the impact of this training on knowledge at a 4–6-week follow-up, and qualitative reports of participants' experiences and strategy application. Beyond interventions, there is also a need for measures specific to pain in people with IDD which can be used in evaluation studies such as the one described above. To start, Genik, Zaretsky, Freedman-Kalchman, & McMurtry (in preparation) have developed a pain-related knowledge questionnaire specific to pain in people with IDD. The most current edition is the Questionnaire for Understanding Pain in Individuals with Intellectual Disabilities - Caregiver Report Revised (QUPID-CR), a 39-item true/false and multiple choice questionnaire which was designed according to pre-existing literature and Chap. 43 of the International Association for the Study of Pain's (IASP) core curriculum (Pain Issues in Individuals with Limited Ability to Communicate Due to Cognitive Impairment; [110]).

With respect to relevant resources, it is critical to also acknowledge parents and other primary caregivers as key information sources when it comes to pain [102, 111]. Understanding factors such as individual's baseline and history, both pain and otherwise, is necessary in order to examine a person's behavior within a given context [103]. Although there are some pain assessment measures developed for health-care contexts in particular, these may not be appropriate or feasible for community-based settings. Moving forward, it will be critical to adapt and develop resources that better suit non-health-care contexts and facilitate communication between parents and other secondary providers. An example of a resource currently under development and preliminary evaluation is the empirically informed Caregiver Pain Resource (Genik, Millett, & McMurtry, in preparation). This resource is designed for parents to record important information about their child's pain, pain expression, and pain management so that secondary caregivers have pertinent information readily available.

Initial results from a feasibility and usability study are overwhelmingly positive.

Continuing Issues and Hope for the Future

Children with IDD should be receiving the same standard of care as their typically developing peers regardless of their ability to express pain. Any painful event (e.g., bone break), condition (e.g., constipation, osteoporosis), or procedure (e.g., surgery) should be approached with the same care that would otherwise be given. Given persisting perspectives regarding pain sensitivity and indifference, further research needs to explore the pain thresholds of individuals with IDD using high-quality research designs to provide as strong as possible evidence one way or the other. High-quality research is needed in every avenue of this nascent field and there is an especially large gap in research including participants with severe and profound IDD. Pain is an all too common experience for individuals with IDD and it is of critical importance that we understand it as well as we can scientifically improve clinical care for children who may be suffering in silence.

Summary

In this chapter, we discussed the approaches and issues associated with the study of pain in IDD, specifically including:

- assessing sensory function and pain thresholds
- using observational assessments of pain intensity
- relying on proxy-report for pain assessment
- testing biomarkers of pain/inflammation

Knowledge translation initiatives are needed to further implement pain assessment practices and improve provider/caregiver education specific to pain in this population. Ongoing challenges include the bias or belief that individuals with IDD have reduced sensitivity to pain. Further research is needed to explore pain thresholds using high-quality research designs. In the meantime, providers and caregivers are encouraged to provide the same pain-relieving care that would otherwise be given.

Multiple Choice Questions

1. Ideally, a pain treatment plan would include the following steps:
   (a) Assessment, psychological testing, new medications
   (b) **Assessment, diagnosis, treatment, follow-up, communication**
   (c) Diagnostic testing, ketogenic diet, acetaminophen, follow-up assessments
   (d) Treatment, communication, blood tests, PET scan
2. Which of the following pain assessments were created specifically for individuals with IDD?
   (a) Paediatric Pain Profile (PPP)
   (b) Non-communicating Children's Pain Checklist–Revised (NCCPC-R)
   (c) Faces Legs Activity Cry Consolability Scale–Revised (r-FLACC)
   (d) **All of the above**
3. Which of the following is true regarding parent proxy report of pain in IDD?
   (a) Parents always know when their child is in pain
   (b) **Parent proxy report may be influenced by factors other than their child's pain**
   (c) Parents are better at detecting pain in their child with IDD compared to their siblings
   (d) None of the above
4. Biomarkers alone will readily support the inference that "the individual is in pain"
   (a) True
   (b) **False**
5. Which of the following statements are true with regard to caregiver roles in pain in IDD?
   (a) Programs to improve caregivers' knowledge on pain in IDD are successful
   (b) Caregivers play an important role in the pain assessment and management process
   (c) Disability-related beliefs held by caregivers may impact care decisions
   (d) **All of the above**

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Pharmacology, Psychopharmacology, and Adverse Drug Reactions

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Learning Objectives

The goals of this chapter are to provide readers with an overview of basic psychopharmacology, and how these principles, apply to the treatment of children with behavioral and psychiatric disorders. Upon the completion of this chapter, the reader should demonstrate the following:

1. A working knowledge of pharmacogenomics, the relationship between pharmacogenetics and pharmacokinetics and pharmacodynamics, and the pathophysiology of childhood behavioral and psychiatric disorders.
2. Apply the concept that psychiatric disorders are neurodevelopmental in nature and how this understanding, influences treatment selection and clinical monitoring.
3. A capacity to apply the activity, variability, and specificity of the CYT P450 systems to variations in drug metabolism, drug–drug interactions, and risk factor for adverse drug reactions.
4. Describe the role of various diagnostic approaches plays in the selection of psychotropic drugs.
5. Discuss the issues related to age of onset, comorbidities, and genetic vulnerability play in shaping the clinical course of psychiatric disorders and variability of treatment response.
6. Describe the roadblocks in developing precision treatment principles for psychiatric disorders.

Highlights

- The history of psychopharmacology began serendipitously with the application of certain medications with neurobehavioral side effects to the treatment of primary psychiatric disorders. These decisions helped initiate modern psychopharmacology.
- The development of psychotropic medications contributed to our deeper understanding of the neurobiology of many chronic, neuropsychiatric disorders.
- The same basic principles of absorption, distribution, metabolism, and the need to search for mechanisms of action apply to psychotropic medications.
- New developments in the neuroscience are changing our understanding of behavior disorders and psychopathology. These changes will influence future approaches to diagnosis and the developmental of novel treatments.
- The relationships between genetic risk and metabolic disturbances, neurodevelopmental nature of many psychiatric disorders, and the complex interrelationship between neurodevelopmental disorders (intellectual disability, autism spectrum, and many neuropsychiatric disorders) and psychiatric disorders require rethinking of our usual approaches to diagnosis and treatment approaches.
- At present, clinicians continue to struggle with issues related to incomplete treatment responses, low remission rates, frequent need for augmentation and multi-drug regimens, and a lack of disease-altering cures. These frustrations in part relate to problems associated with the heterogeneity of most neurodevelopmental and psychiatric disorders. We still have a lot to learn.

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Introduction

The modern era of psychopharmacology began when astute clinicians realized the potential effectiveness of pre-anesthetic and anti-tuberculosis agents in the treatment of major psychiatric disorders. Over the next 60 years, psychopharmacologists formulated several schemas for classifying psychotropic drugs. The most enduring ideas focused on syndrome specificity (antipsychotics, antidepressants, mood stabilization, etc.), preferential receptor binding sites (dopamine, norepinephrine, etc.), and principle functions (antagonists, agonists, partial agonists, reuptake sites, etc.).

In recent decades, there was a growing disconnect between the pace of developments in the neuroscience of mental disorders and the emergence of new or novel psychotropic drugs. Although the costs of drug development are high, many promising drugs leave the laboratory (animal models) only to fall by the wayside during the U.S. Food and Drug Administration (FDA) approval process. It appears that novel compounds run head on into the complexity of human neurobiology, and our limited understanding of the pathophysiology of psychiatric disorders. As clinicians many of us struggle with pharmacological interventions that still produce remission rates in the 33% range, clinical improvement in a large part of the remainder, and smaller percentage of patients who are either non-responders or worsen on available treatment protocols (except perhaps for clozapine).

This chapter briefly explores two aspects of this problem: our incomplete understanding of both the pathophysiology of mental disorders (syndromal heterogeneity), and the equally complex world of pharmacogenomics, pharmacogenetics, pharmacokinetics, and pharmacodynamics of psychotropic medications. As we shall see, ongoing brain development and maturation during childhood and adolescence adds a new set of complexities to these problems [1, 2].

Searching for solutions to these problems require us to explore new ideas in neuropharmacology and psychiatric diagnostics. This task is not a simple, straightforward one, especially in an era of rapid advances in the neurosciences, genomics, molecular neuropharmacology, and developmental pathophysiology. Our goal is to distill and translate these concepts for practicing clinicians, and address the complexities of precision medicine in patients with neurodevelopmental psychiatric disorders.

Psychopharmacology as a Subset of Basic Pharmacology

Pharmacology is the study and application of medicinal compounds to the treatment of disease states. Psychopharmacologists adopt the core principles of pharmacogenomics, pharmacogenetics, pharmacokinetics, and pharmacodynamics to the treatment of neurobehavioral and psychiatric disorders.

From the 1950s onward, our biological models of mental disorders developed in tandem with our emerging psychopharmacological treatments. For example, the dopamine hypothesis of schizophrenia (SCZ) emerged from studies that demonstrated the psychotomimetic effects of psychostimulants (blocking reuptake deactivation and facilitating dopamine release), the antipsychotic effects of dopamine antagonists (neuroleptics), and the role of altered dopamine/acetylcholine ratios in the emergence of pseudo-Parkinsonian (extrapyramidal side effects) and tardive dyskinesias. Attempts to minimize extrapyramidal side effects (EPS) (labeled pseudo-Parkinsonism at the time) and enhance treatment efficacy led to the development of second-generation antipsychotics that included serotonergic, adrenergic, antihistaminic, and additional dopamine receptor antagonists [1, 3, 4].

The original, standard model for classifying psychotropes focused on specific syndromes (antidepressant or antipsychotic, etc.), agonist and antagonist effects for specific neurotransmitters selective serotonin reuptake inhibitors (SSRIs), dopamine blockers, adrenergic), or receptor subtypes (postsynaptic, presynaptic, neurotransmitter reuptake sites, multiple neuron binding). Since then, the list expanded to include neurohormones and neuropeptides, neuro-inflammatory cytokines, and peptide receptors; subfamilies of receptor subtypes; neuromodulators; ion transport channels (ionotropic); and second messenger systems (metabotropic) [3, 5–7].

Yet, when compared to treatments developed for somatic disorders, it quickly becomes apparent that syndrome-specific treatment of psychiatric disorders can neither be precisely characterized nor targeted treatments specified (precision) to the level seen in the treatment of infectious diseases or cancer. Part of the problem is our incomplete understanding of brain development, neuroplasticity and synaptic function, myelination, interactive specialization, and coherence, and how these forces interact with developing psychopathology and treatment across the life cycle. Only in the last few decades have neuroscientists had access to the genetic tools and functional neurophysiological and neuroimaging technologies of sufficient power to begin disentangling the mosaic of developmental pathophysiology [3, 5, 6, 10, 11].

As a result, behavioral health providers frequently operate in the domains of clinical history, observations and physical and mental status examinations, and psychological/neuropsychological testing. Even though new diagnostic tests and technologies are available, their use is limited. For most clinicians, generating psychiatric diagnoses remains in the realm of descriptive phenomenology in spite of ongoing problems with diagnostic heterogeneity variability in treatment efficacy [12, 13]. In the past decade, the application of pharmacogenetics and pharmacokinetics emerged as part of an effort to enhance treatment efficacy, increase remission rates, minimze adverse side effects attributable to variability in ratrs of metabolism, minimize

polypharmacy, minimize drug–drug interaction effects, and decrease the risk for adverse drug reactions [14, 15] (discussed in greater depth in Appendix 1).

Pharmacogenomics

Pharmacogenomics addresses the influence of the human genome on drug responses. These influences include genomic effects on the targeted receptors, intracellular mechanisms of action, and other syndrome-specific, pathophysiological characteristics of the disease process [11, 18]. The Human Genome Project birthed a renewed interest in pharmacogenomics. At one level, geneticists could focus on psychiatric disorders as complex phenotypes with polygenic and epigenetic signatures. The study of single nucleotide polymorphisms (SNPs), copy number variants (CNVs), mechanisms of gene regulation, and epigenetics expanded our view of pharmacokinetics, pharmacodynamics, as well as the deep structure of genetic risk. These findings also prompted discussions about linking new genetic data on psychiatric disorders to pharmacogenetics of treatment models [19–21].

The focus on neurodevelopmental disorders like autism spectrum disorder (ASD) and intellectual disability/intellectual developmental disorder (ID/IDD) as behavioral phenotypes took on a new meaning. Originally, behavioral phenotypes were limited to behavior, temperament, emotional dysregulation, and cognitive deficits associated with specific genetic disorders associated with ID/IDD [13]. Original discussion focused on a direct link between genes, brain, and behavior, but there were challenges to the universality of these proposed linkage phenotypes. It was apparent that there was still variability in the expression of these genetic disorders, suggesting the effects of genetic variation due to gene × environment interactions, and extra-nuclear, transcription/translation, and other local modes of gene regulation. The certainty of behavioral phenotypes contributed to a more probabilistic model of brain development and behavioral phenotypes [22].

Yet, these new data did not produce a seamless transition for behavioral health practitioners. Many struggle to integrate data from Genome and Exome Scans, mitochondrial genetics and physiology, glial function, and neuroimmunology/role of inflammatory cytokines into our conceptual and treatment models for major psychiatric disorders [5–8, 25].

Pharmacogenetics (See Appendix 1)

Pharmacogenetics narrows the focus of study to inherited genetic differences in drug metabolic pathways that affect individual responses to medicinals, both in terms of therapeutic and adverse effects [11].

Pharmacokinetics

Pharmacokinetics encompasses the absorption, distribution, and transportation of a drug to its target receptor. This process begins with gut absorption (active and passive transport), first pass metabolism by the liver, and transportation and binding to the specific target sites. Once the task of receptor binding is complete, the next pharmacokinetic step involves its elimination via biotransformation and excretion. Drug metabolism involves the biotransformation of medicinals through a complex sequence of enzymatic transformations of lipophilic (fat-soluble) into hydrophilic (water-soluble) compounds for excretion. The process involves oxidation, reduction, and hydrolysis, which involves the complex oxidative cytochrome P450 (CYT P450) pathways. The CYT P450 system includes a diverse system of enzymes that metabolize multiple classes of exobiotics, hormones, and pharmaceuticals [3, 9, 10].

The CYT P450 system displays considerable genetic variability in terms of enzymatic activity, and sensitivity to induction and inhibition by other agents. These variations in enzymatic activity (metabolic rates) help determine the pharmacological half-lives (T1/2) as well as dose–response curves, kinetic profiles (serum drug level dependent or independent), drug–drug interactions, and vulnerability to adverse drug events. Many medications require several CYT P450 enzymes and have some of their metabolites active. Unfortunately, these metabolites may also contribute to toxicity and adverse drug events. As noted above, the CYT P450 network may also serve to activate prodrugs that require conversion in order to be effective. Lastly, the variability in the CYT P450 activity has led to confusion among clinicians regarding drug efficacy (very rapid metabolizer), toxicity (slow metabolizers), inhibition (mimicking slow metabolism), and induction (mimicking rapid metabolism and accelerated hormone metabolism) [3, 10, 11, 26].

Pharmacokinetic studies also expanded to include a variety of new metabolic considerations. For example, psychotropic drugs that inhibit CYP P450 may produce toxicity at therapeutic drug levels and increase the likelihood of adverse drug–drug reactions (EPS/akathisia, delirium). In these circumstances, it is prudent to reduce the dose of the psychotropic. All too often, however, clinicians misattribute side effects and adverse reactions as either a loss of effect, a new disorder, or exacerbation of current psychiatric problem. These misattributions frequently lead to unmanageable polypharmacy, increasingly complex drug–drug interactions, and increases in clinical morbidity and mortality [11, 26].

Three pharmacokinetic factors receive less publicity: factors related to rate and extent of absorption, central nervous system (CNS) and neuronal uptake, and degree of binding to transport proteins [26–28] (albumin and alpha glycoproteins). The uptake and/or rejection of anticonvulsant and psychotro-

pic medications are modifiable by *p*-glycoprotein activity [29]. Both can influence multi-drug resistance. The second issue involves the transportation of drug to their targets. Clinically, the extent and intensity of protein binding can influence drug response. In situations where high-level protein binding is prominent, toxicity may occur in spite of normal serum drug levels when a second drug competes for the same binding proteins [3, 4, 10, 29]. In these cases, the serum level may appear normal but the free drug fraction of drug available to act is significantly elevated. The third involves the volume of distribution and lipo- versus hydrophilicity of the drugs [9]. For children, increased hepatic metabolic activity and variable lipid/waters ratio can affect the volume of distribution and bioavailability. A similar problem occurs with bioavailability of lipophilic drugs in obese patients. Fat cells may alter drug distribution and the dose–response curves [9, 10, 30].

Aside from the free drug fraction issue, there is the gray zone between pharmacokinetics and pharmacodynamics. One source is our reliance on augmentation strategies that can affect second messenger systems, or alter intracellular activity without affecting receptor binding and metabolism [3]. Some inducing agents (e.g., carbamazepine, lamotrigine) may induce the metabolism of selected steroids sufficiently to cause a reduction in hormone efficacy. The best example is the accelerated clearance of oral contraceptive pills or progesterone by carbamazepine and the risk of break through bleeding, or unanticipated pregnancy [9, 10].

Bullets

- Drug metabolism is a process that involves absorption, transportation, attachment to receptor sites, and inactivation.
- Pharmacogenetics incorporate gene that influences the CYT P450 system as well as others on sites of biotransformation in drug metabolism.
- The metabolism of many psychotropic medications is a multistep process that involves the CYT P450.
- Drug–drug interactions are a common source of adverse drug reactions.

Pharmacodynamics

Pharmacodynamics deals with the mechanisms of action for medications. This effect frequently dovetails into the complex interactions between drug effects and the pathophysiology of the condition under treatment. Pharmacodynamics now also includes a wealth of new data from molecular biology, neurophysiology, functional neuroanatomy, and pharmacogenetics. These new data have also modified our approach to mental disorders suggesting that we can no longer search for specific "lesions" or a single neurotransmitter. Instead, complex behavioral and mental disorders arise from dysfunctional brain networks with multiple families of neurons and glial cells; a myriad of neurotransmitters and modulators contribute to regional variations in the basic pharmacology of many psychotropic drug–receptor interactions (1, 3, 11 18).

As a molecular neuroscience, pharmacodynamics now encompasses intracellular, molecular pathophysiology of drug action: second messenger systems, gene regulation, local protein transcription, and synaptic neuroplasticity [3, 4]. In addition to the mechanics of neurotransmitter–receptor binding and activation, the neurobiology of most psychiatric disorders also involves changes in neuronal activity related to electrophysiological communication between neurons, and key nodal points connecting to more extensive neuronal networks. This complexity may explain why it is so difficult to drill down on the pharmacodynamics of drug effects with certainty or precision. Variabilities and probabilities are the rules of neuroscience—many syndromes are final common pathways consisting of many patterns of inheritance, and neurobiology, that converge into a heterogeneous disorder like depression, attention-deficit hyperactivity disorder (ADHD), ASD and aggression, self-injury, and cognitive dysfunction [2, 3, 21, 24].

These dynamic changes have special relevance to brain development and maturation during childhood. This is a period when synaptic complexity ebbs and flows, the top-down regulatory function of cortical circuits come on line, and myelination and tract development are contributing to interactive specialization and coherence [1, 3, 31, 32]. These changes contribute to the hierarchical organization and integration of brain networks associated with emerging cognitions, emotional regulation, social intelligences and communication, and multiple executive functions. Children with atypical development experience irregularities in the sequential switching on/off of multiple genes in response to environmental challenges. These atypical neurodevelopmental trajectories serve in predisposing (vulnerability), precipitation (trigger stimuli), and persistence of symptoms and clinical course of behavioral and psychiatric disorders. They also influence treatment response based on variations in sensitivity and adverse response to a range of psychotropic medications [5, 8, 31–33].

Bullets

- Pharmacodynamics deals with the mechanism of action for a specific medication.
- Recent advances in basic neuroscience and new technical advances in intracellular biology provide new insights and treatments for many genetic disorders.
- Treatment of non-responders deserve both pharmacogenetic and a systematic review of diagnosis, pathophysiology, and past treatments.

Precision Medicine

Precision medicine is the process of defining and applying specific genetic biomarkers to the treatment of disease states. Some years ago, the process involved the introduction of identifying bacteria in culture and then assessing the sensitivity of these pathogens to specific antibiotics. The modern era of chemotherapies and biological treatments focuses on directing treatment to specific cell surface antigens, pathogenic antibodies, and defeating the offending pathogens from disguising themselves or deactivating the immune responses. These approaches focus on the gene markers, or various innate and the adaptive components of inflammatory and immune systems. The first step involves molecular biological approaches to diagnosis. The second component of this approach focuses on increasingly specific pharmaceuticals, biologicals, and chemotherapies. This level of precision also involves pharmacogenetics and pharmacokinetic principles in an effort to design treatments to the individual patient [28, 35].

Bullets

- Pharmacogenomics is an overarching concept that deals with the genetic forces that affect both drug effects and the pathophysiology of the disease state under treatment.
- Pharmacogenetics narrows the focus of study to inherited genetic differences in drug metabolic pathways.
- Pharmacokinetics involves the processes of absorption, distribution, and transportation of a drug to its target receptor.
- Pharmacodynamics deal with mechanisms of action for medications, but also dovetails into the underlying pathophysiology of the medical condition under treatment.
- Precision medicine involves pharmacogenetics and pharmacokinetics in the development of individualized treatment.

Classifying Psychiatric Disorders

There have been many paradigm shifts during the relatively short history of modern psychiatry. The most significant step was the introduction of the diagnostic statistical manual (DSM-III) in the early 1980s. The DSM-III replaced the psychodynamic foundation for diagnosis with a multi-axial diagnostic system based on a categorical/descriptive, phenomenological methodology for classifying psychiatric syndromes. Subsequent upgrades of DSM continued to de-emphasize etiopathogenesis and focus on the need for improved descriptive categorization in order to enhance communication and reliability through the use of a common diagnostic language. A similar model was the approach used by the National Association for Dual Diagnosis to adapt these criteria for patients with ID/IDD. The results of these efforts were the Diagnostic Manual-Intellectual Disability 1 (DM-ID-1) published in 2007 and the follow-up DM-ID-2 in 2016 [12, 13].

During the years required for publication, there were growing concerns by researchers (geneticists and neuroscientists) about the heterogeneity and basic descriptive/categorical approach of psychiatric diagnosis. The descriptive approach troubled neuroscientists and neuropharmacologists in large part due to the growing evidence that there were shared genetic and neurobiological markers between discrete disorders. In addition, there were high rates of comorbidity disorders, and concerns about the relative lack of syndrome and neuropharmacological specificity for increasingly sophisticated research studies. Many clinicians also raised concerns about the variability in disorders like major depression heterogeneity based on age of onset, severity, presence of comorbidities, variable longitudinal and clinical courses, and incomplete treatment response [5, 34, 36–39].

One of the most persistent set of comorbidities involved neurodevelopmental disorders, especially autism spectrum disorder (ASD) and intellectual disability/intellectual developmental disorder (ID/IDD). Children with ASD and ID/IDD display higher rates of psychopathology (ADHD, anxiety and mood disorders) than are present in the general or neurotypical populations, in spite of under-diagnosis [13, 15–17]. Following factors emerged as major concerns:

1. The baseline exaggeration of disruptive, aggressive, or self-injurious target behaviors frequently overshadowed the core symptoms of a psychiatric disorder.
2. Recognition of psychiatric disorders as complicated patterns of cognitive and behavioral disorganization, especially during periods of trauma and distress.
3. The vulnerability to regression in adverse settings was frequently misattributed to severe psychopathology.
4. The overprescribing of psychotropes for "challenging behaviors" or emotional dysregulation (irritability) without an adequate assessment, ongoing environmental/behavioral interventions, or a specific psychiatric diagnosis [15, 41].

The presence of severe ASD ± ID/IDD also required revisions in the biopsychosocial model, especially existing stress–diathesis concepts. For many patients, behavioral and mental disorders represent an imbalance between genetic risk, and adaptive skills and the severity of precipitating factors. In general, limited adaptive skills and lower resilience

when accompanied by difficult temperament, or disorganized attachment needs, increase the risk for psychopathology. These vulnerabilities are compounded by the presence of primary genetic, metabolic, and neurological disorders associated with this population and increase their risk for developing severe mental illness and a more limited treatment response [13, 17, 23].

Genome-wide scans further suggested that many polymorphisms (single nucleotide polymorphisms) with small effect sizes, larger gene irregularities (copy number variants) with greater effect sizes, and pleiotropic influences on phenotypic expression did not match up exclusively with specific psychiatric syndromes. In addition, these neurodevelopmental disorders shared many genetic markers with several major psychiatric disorders, suggesting that temperament and patterns of attachment appear across several diagnostic categories [14, 19, 20].

As a result, researchers turned to the Research Domain Criteria (RDoC), intermediate and biological endophenotpying strategies, shifting the focus of future neuropharmacological research from syndrome diagnosis to shared endophenotypes that resembled neurobiologically sophisticated versions of temperament traits [36–39]. Some clinicians had concerns about the lack of specificity and heterogeneity that contributed to low rates of clinical remission, higher numbers of partial responders, and a subpopulation of treatment non-responders. Yet many had problems with the RDoC, feeling it was unworkable for clinical practitioners.

It appeared that some form of algorithmic, phenomenological endophenotyping (subtyping) that included patterns of comorbidity, similar genetic markers, and the shared efficacy of psychotherapies and psychotropic medications could be useful in an era of evidenced-based medicine [19, 34, 36]. Unfortunately, earlier drug studies excluded patients with neurodevelopmental disorders and many treatment approaches were extrapolated from studies with neurotypical children. New data suggest they are more inclusive but unfortunately, many of these treatment studies concentrate on heterogeneous psychiatric disorders or nonspecific behavioral disorders like agitation, irritability, aggression, self-injury, and social withdrawal [4, 14].

As noted earlier, the misattribution of side effects and adverse drug reactions to syndromal worsening contributed to a pharmacological arms race—the simultaneous prescribing of multiple drugs in the same class (multiple antipsychotics) or in empirically driven combinations (40, 42,). As alluded to throughout this chapter, behavioral disturbances, psychiatric disorders, and the use of psychotropic drugs do not occur in a vacuum and that systemic and comprehensive interventions are necessary for positive outcomes [40, 42].

The extraordinary volume of off-label prescribing is in part due to the limited number of FDA-approved psychotropic drugs for individuals with neurodevelopmental disorders. For example, the antipsychotic drugs risperidone and aripiprazole have an FDA indication for treating irritability and related aggressive behaviors associated with autism spectrum disorders (ASD). Similar data justify the use of other antipsychotic, antidepressant, and mood stabilizer for nonspecific emotional dysregulation and disruptive behaviors. To date, we have few drugs that can correct the underlying brain dysfunction associated with neurodevelopmental disorders. It may turn out that these drugs are more useful earlier during brain development and maturation, but also carry greater risks for affecting subsequent development [3, 8, 42].

Below are the commonly prescribed medications by class, mechanism of action (receptor agonist or antagonist, reuptake inhibitor, etc.), indications for use, major side effects, and common drug–drug interactions. Listing medications by class is based on conventional categorization, and these classes describe the intended effects of the medication in the majority of cases (e.g., antidepressants), but it is important to note that many drugs also have indications for other diagnoses or purposes.

Several issues stand out in Table 44.1:

1. There are many factors affecting drug response. Some are pharmacokinetic while others relate to complexities of pharmacodynamics and pathophysiology of presenting problems.
2. These psychotropic drugs are labeled and frequently prescribed based on mechanism of action or for specific disorders. Most, however, are used as multipurpose drug for a variety of related diagnoses.
3. The mechanisms of actions are far more complex than originally considered. Recent research suggests that their efficacy may be related to shared genetic, neurophysiological markers and other pathophysiological features that are not specific to any specific syndrome.
4. Side-effect profiles may also differ among psychotropic drugs in the same family. For example, SSRIs differ in terms of CYT P450 metabolic pathways, clinical activity of metabolites, and differences in binding site profiles.
5. Status as inhibitors or inducers of CYT P450 activity affects hormone metabolism, serum drug level, and drug–drug interactions when multiple drugs are used (polypharmacy).
6. Adverse drug reactions relate to idiosyncratic factors, racial/ethnic variations, and chronic use. Antipsychotic drugs block dopamine receptors and play a key role in the development of extrapyramidal side effects. In vulnerable patients, chronic use contributes to late onset dyskinesias (tardive dyskinesias) and about 20% of these affected individuals may develop chronic abnormal movements [1, 3, 4, 26, 30, 40, 42–49].

(See Appendix 1 for more details)

Table 44.1 Psychotropic medications, uses, mechanism of action, interactions and side effects

Drug class	Use(s)	Mechanism of action	Common or notable interactions	Major or common side effects
Antidepressants	FDA-issued black box warning for increased risk of suicidality in patients <24 years old			
Selective serotonin reuptake inhibitors (SSRIs)	Depressive disorders (MDD), anxiety disorders (GAD, panic disorder, social anxiety disorder), PTSD, obsessive compulsive disorder (OCD), Bulimia nervosa, premenstrual dysphoric disorder	Block transporters for serotonin; change receptor sensitivity; up/down regulation of receptors	Medication interactions: some atypical antipsychotics compete for the same CYP enzymes (especially risperidone, aripiprazole), Tamoxifen (with SSRIs that inhibit CYP 2D6), thioridazine (due to cardiac conduction: prolonged Q wave-T wave interval (QTc) prolongation), pimozide, MAOIs or other SSRI/SNRIs (risk of serotonin syndrome), carbamazepine, benzodiazepines (most notable interaction with fluvoxamine) Other products: cigarettes (most notable interaction with fluvoxamine)	Activation and/or insomnia (especially fluoxetine, sertraline), discontinuation syndrome when abruptly stopped (especially paroxetine), hyponatremia including risk of SIADH, increased bleeding risk, increased risk of bone fractures, QTc prolongation (citalopram), sexual dysfunction (especially delayed ejaculation in men or anorgasmia in either sex), weight gain
Serotonin norepinephrine reuptake inhibitors (SNRIs)	Depressive disorders (MDD), anxiety disorders (GAD), pain syndromes	Block transporters for norepinephrine and serotonin; change receptor sensitivity; up/down regulation of receptors	Medication interactions: MAOIs or other SSRIs/SNRIs (risk of serotonin syndrome); use of proton pump inhibitors affects absorption of duloxetine May produce a false-positive on urine drug tests for amphetamines (especially venlafaxine)	Abnormal or increased bleeding, activation, contraindicated in the acute recovery period after myocardial infarction, decreased appetite, discontinuation syndrome when abruptly stopped (especially venlafaxine), dry mouth, hypertension (especially venlafaxine), hyponatremia including risk of SIADH, insomnia
Tricyclic antidepressants (TCAs)	Depressive disorders (MDD), OCD, childhood enuresis, off-label uses for neuropathic pain, migraine	Block transporters for norepinephrine, dopamine and/or serotonin; change receptor sensitivity; up/down regulation of receptors; blockade of voltage-gated ion channels	Cisapride (due to prolonged QTc interval), MAOIs or other SSRIs or SNRIs (risk of serotonin syndrome), haloperidol and phenobarbital interact with clomipramine	Arrhythmia (especially in TCA overdose), contraindicated in the acute recovery period after myocardial infarction, diarrhea, drug rash and hypersensitivity, dry mouth, hypertension, hypotension and dizziness, nausea, QTc prolongation, urinary retention, vomiting
Monoamine oxidase inhibitors (MAOIs)	Depressive disorders (both FDA indicated for treatment resistant MDD and off-label uses), off-label use in anxiety disorders, adjunctive therapy in Parkinson disease	Enzyme inhibition alters availability of monoamines	Dietary interactions: patients taking MAOIs must avoid foods high in tyramine (aged or fermented products, certain meats, chocolates, red wine) Medication interactions: anesthetics (vasoconstriction, especially with epinephrine, can cause blood pressure changes and crisis), carbamazepine, decongestants and stimulants (risk of hypertensive crisis), fentanyl and other opioids, IV methylene blue, linezolid, meperidine, other MAOIs/SSRIs/SNRIs due to risk of serontonin syndrome when used in combination, S-adenosyl-L-methionine (SAM-e), Saint John's wort, triptans, tryptophan	Discontinuation syndrome when stopped abruptly, dizziness, dry mouth, hepatotoxicity, insomnia, lightheadedness, local skin reaction (for those using a transdermal patch), seizures, sexual dysfunction, tyramine crisis (see comments on dietary interactions), urinary retention, weight gain or loss
Others (bupropion, mirtazepine, trazodone)	Depressive disorders (MDD), smoking cessation (bupropion)	Block transporters for norepinephrine, dopamine and/or serotonin; change receptor sensitivity; up/down regulation of receptors; blockade of voltage-gated ion channels	Medication interactions: MAOIs or other SSRIs or SNRIs (risk of serotonin syndrome) Bupropion: May produce a false-positive on urine drug tests for amphetamines	Activation (especially bupropion), discontinuation syndrome when abruptly stopped, drowsiness (mirtazapine, trazodone), dry mouth, insomnia (bupropion), lowered seizure threshold (bupropion), priapism (trazodone), QTc prolongation (trazodone), weight gain (especially mirtazepine), weight loss (bupropion)

(continued)

Table 44.1 (continued)

Drug class	Use(s)	Mechanism of action	Common or notable interactions	Major or common side effects
Anti-manic/mood stabilizer	Antiepileptics (AEDs) can increase the risk of suicidal thoughts or behavior in patients taking the medication for any reason			
Lithium	Bipolar mania	Binding to voltage-gated ion channels, intracellular changes/modulation of signal transduction pathways, up/downregulation of receptors	Angiotensin converting enzyme inhibitors and angiotensin receptor blockers, calcium channel blockers, diuretics, metronidazole, NSAIDs, products containing caffeine, products containing theophylline, can cause encephalopathic syndrome when combined with haldol	Acne, chronic kidney disease, nausea/vomiting/diarrhea, fatigue/sedation, hypercalcemia and hyperparathyroidism, hyponatremia, impaired thyroid function, tremor, weight gain
Valproic acid	Bipolar mania, seizure disorders, migraine	Binding to voltage-gated ion channels, intracellular changes/modulation of signal transduction pathways	Antipsychotics (chlorpromazine, haloperidol), barbiturates, benzodiazepines, estrogen, other antiepileptics (phenytoin, phosphenytoin, carbamazepine, lamotrigine, ethosuximide), some anti-infective agents (carbapenems, rifampin)	Blood dyscrasias, encephalopathy, hepatic failure, increased risk of neural tube defects if used in pregnancy, multiorgan hypersensitivity reaction is possible, pancreatitis, sedation, weight gain
Lamotrigine	Bipolar disorder, epilepsy	Binding to voltage-gated ion channels (sodium), intracellular changes/modulation of signal transduction pathways	Anti-infective medications (atazanavir, ritonavir, rifampin), barbiturates, estrogen and progesterone, other antiepileptics (carbamazepine, phenytoin, fosphenytoin, valproic acid)	Blood dyscrasias, hemophagocytic lymphohistiocytosis (HLH), hypersensitivity reactions including SJS and TEN, multiorgan hypersensitivity reaction is possible, suicidal ideation can occur
Carbamazepine	Epilepsy, trigeminal neuralgia, off label use in bipolar disorder	Binding to voltage-gated ion channels (sodium), intracellular changes/modulation of signal transduction pathways	Antipsychotics (specifically aripiprazole, brexpiprazole, risperidone), buspirone, calcium channel blockers, estrogen and Tamoxifen, guanfacine, other antiepileptics (phenytoin, fosphenytoin, lamotrigine, topiramate, valproic acid), protease inhibitors, theophylline derivatives, vilazodone, vortioxetine, warfarin Should be taken with food	Aplastic anemia and agranulocytosis, hyponatremia, multiorgan hypersensitivity reaction is possible, Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) especially with HLA-B*1502 genotype, suicidal ideation can occur
Oxcarbazepine	Epilepsy, off label use in bipolar disorder	Binding to voltage-gated ion channels (sodium), intracellular changes/modulation of signal transduction pathways	Barbiturates, estrogen and progesterone, other antiepileptics (phenytoin and fosphenytoin)	Blood dyscrasias, bone changes, hypersensitivity reactions including SJS and TEN, hyponatremia, hypothyroidism, multiorgan hypersensitivity reaction is possible, suicidal ideation can occur
Gabapentin	Postherpetic neuralgia, epilepsy; off-label use for pain, migraine, as a mood stabilizer or in treatment of anxiety	Some binding at voltage-gated ion channels (calcium), mechanism largely unknown	Can potentiate CNS depression when combined with other depressants, recommended to wait 2 hours after Maalox before taking due to change in absorption	Dehydration, discontinuation syndrome if stopped abruptly, fatigue or malaise, hypertension or hypotension, paresthesias, purpura or less frequently blood dyscrasias can occur, tremor, vertigo
Topiramate	Seizure disorders, migraine prophylaxis, off label psychiatric use as a mood stabilizer	Binding to voltage-gated ion channels, intracellular changes/modulation of signal transduction pathways	Lithium (need for more frequent monitoring of lithium levels if using high-dose topiramate), oral contraceptives (decreased efficacy of OCP)	Acute myopia and secondary angle closure glaucoma, cognitive dysfunction, kidney stones, metabolic acidosis, oligohydrosis and hyperthermia, suicidal ideation can occur, weight loss
Antipsychotics	FDA-issued black box warning for increased risk of death in elderly patients with dementia-related psychosis treated with antipsychotics			

Typical/First generation	Acute intermittent porphyria (chlorpromazine), agitation (droperidol), bipolar disorder (mania: chlorpromazine), generalized nonpsychotic anxiety (prochlorperazine, trifluoperazine), hyperactivity (haloperidol, chlorpromazine), intractable hiccups (chlorpromazine), nausea and vomiting (chlorpromazine), schizophrenia, severe childhood behavioral problems (chlorpromazine, haloperidol), Tourette syndrome (haloperidol, pimozide)	Effect availability of monoamine neurotransmitters, specifically blockade of dopamine; blockade of M1 muscarinic cholinergic receptors and/or H1 histamine receptors (especially "typical" or "first generation" antipsychotics); activity at serotonin receptors (both partial agonist and antagonist action)	Potentiation of CNS depression when combined with other depressants (opiates, barbiturates, alcohol), increased risk of NMS when combined with other neuroleptics Specific interactions: propranolol, pindolol, fluoxetine, paroxetine, fluvoxamine (will increase thioridazine levels and potentiate effects including prolonged QTc); carbamazepine (induces metabolism of thiothixene); citalopram or escitalopram (contraindicated with pimozide); thiazide diuretics (accentuate orthostasis); protease inhibitors; azole antifungals; grapefruit juice (for antipsychotics that use CYP 3A4 system)	Blood dyscrasias, cardiometabolic risks (dyslipidemia, metabolic syndrome), D2 blockade can cause worsening of some negative symptoms in schizophrenia (apathy, decrease in interest or motivation for pleasurable activities), dry mouth and constipation (caused by anticholinergic properties), extrapyramidal symptoms (dystonic reactions, akathisia, rigidity), hyperprolactinemia, lowered seizure threshold, neuroleptic malignant syndrome, orthostatic hypotension and falls, ocular changes (chlorpromazine), QTc prolongation and Torsades de pointes, skin pigmentation (chlorpromazine), tardive dyskinesia, weight gain
Atypical/second generation	Adjunctive treatment of depressive disorder (aripiprazole), agitation associated with schizophrenia (aripiprazole, olanzapine, ziprasidone), bipolar disorder (mania: quetiapine, aripiprazole; mixed/manic: aripiprazole, asenapine, olanzapine, risperidone, ziprasidone; maintenance: olanzapine, quetiapine, ziprasidone; depression: lurasidone, olanzapine/fluoxetine combination), irritability or aggressive behavior associated with autism spectrum disorder, schizoaffective disorder, schizophrenia, Tourette syndrome (aripiprazole), treatment resistant depression (olanzapine/fluoxetine combination)	Effect availability of monoamine neurotransmitters, specifically blockade of dopamine; blockade of M1 muscarinic cholinergic receptors and/or H1 histamine receptors; activity at serotonin receptors	CYP3A4 inducers or inhibitors (antivirals, azole antifungals, carbamazepine, clarithromycin, diltiazem, erythromycin, phenytoin, rifampin, St John's wort, verapamil), CYP2D6 inducers or inhibitors (fluoxetine, paroxetine, quinidine), divalproex sodium (may require dose adjustment of the antipsychotic), increased risk of NMS when combined with other neuroleptics, potentiation of CNS depression when combined with other depressants (opiates, barbiturates, alcohol), potentiation of hypotension in patients taking antihypertensive agents, omeprazole (increases olanzapine clearance) Note: some must be taken with food (lurasidone, vilazodone, ziprasidone)	Blood dyscrasias (notably agranulocytosis associated with clozapine), body temperature dysregulartion, cardiometabolic risks (dyslipidemia, metabolic syndrome), D2 blockade can cause worsening of negative symptoms in schizophrenia (apathy, decrease in interest or motivation for pleasurable activities), dry mouth and constipation (caused by anticholinergic properties), dysphagia, eosinophilia (clozapine), extrapyramidal symptoms (dystonic reactions, akathisia, rigidity), hyperprolactinemia, lowered seizure threshold, myocarditis (clozapine), neuroleptic malignant syndrome, QTc prolongation, suicidal ideation can occur, tardive dyskinesia, weight gain
Anxiolytics	Short-term management of anxiety, panic disorder, seizure disorders (acute abortive treatment), skeletal muscle spasm, treatment of acute effects of alcohol withdrawal	Facilitation of GABA activity	FDA-issued black box warning for sedation, coma and death when combining benzodiazepines and opiates; CYP 3A4 inducers or inhibitors, potentiation of CNS depression when combined with other depressants (opiates, barbiturates, alcohol, antipsychotics, sedative hypnotics); divalproex sodium (may require dose adjustment of the anxiolytic), phenytoin	Anterograde amnesia, coma, dependence, fatal overdose, hypotension, paradoxical reaction causing agitation in some patients, respiratory depression, sedation, withdrawal reactions including seizures

Bullets

- Drug metabolism is a complex process with significant clinical impacts.
- The side-effect profile depends on both pharmacokinetic and pharmacodynamic effects.
- The traditional classification of psychotropic drugs is slowly changing along several domains.
- For children on psychotropic drugs, close monitoring is a necessity.

Summary, Synthesis, and Conclusions

The translational neurosciences, genetics, and molecular neuropharmacology are changing many of our basic assumptions about both psychiatric diagnosis and psychopharmacology. These new developments suggest that we need to rethink of models of drug classification [2, 3]; the relationship between pharmacogenetics, pharmacokinetics, and pharmacodynamics and treatment efficacy; and approaches to the diagnosis of behavioral and psychiatric disorders [20, 21]. One source of motivation for these changes arises from the realization of low remission rates, high rates of polypharmacy and augmentation strategies, and concern about the many problems associated with novel psychotropic drug development that plague both researchers and clinicians. [4]. Paradoxically, these frustrations occur against the backdrop of rapid advances in psychiatric genetics, functional neuroimaging, neurophysiology and neuropharmacology, and a growing desire to advance the cause of precision medicine in developmental neuropsychiatry.

Serendipity best describes the origins of psychopharmacology. Beginning in the 1950s, the psychopharmacological revolution began with astute clinicians, clinical observations that some drugs had unanticipated effects, and then an intuitive leap that led to drug trials from some of our most devastating psychiatric disorders. From these humble beginnings, later generations of neuropharmacologists expanded the field by classifying psychotropes based on their efficacy in families of disorders (anti-anxiety, antidepressant, or antipsychotics drugs, or mood stabilizers [syndrome selectivity]) and their ability to alter the activity of neurotransmitters [1]. The next step involved connecting the dots and formulating hypotheses about specific neurotransmitters as causally related to specific psychiatric disorders. Since that time, the development and marketing of new psychotropic drugs expanded the number of receptor agonists and antagonists, partial agonists, and reuptake inhibitors of specific families of neurotransmitters [3].

Researchers soon realized that we are living in a zoo filled with neurotransmitters, receptors, and mechanisms of action and need some reorganization in our methods of classifying psychotropic medications. Zohar et al. [2] proposed a neuroscience-based nomenclature that did not rely on diagnosis but focused instead on pharmacological domains (neurotransmitters/modulators, neurohormones, and ion channels) and modes of action (enzyme inhibitor, modulator, ion channel blocker, enhanced neurotransmitter release, allosteric modulation, agonist/antagonist/partial agonist and reuptake inhibitors). This approach accommodates many of our current ideas about neuropharmacology, but does not directly address pharmacogenetics/pharmacokinetics or the universe of neuropeptides, diffusible neurotransmitters (CO, NO), ionotropics and metabotropics, second messenger and intracellular systems, and gene regulation [2, 3].

There is a renewed interest in exploring ways to increase the specificity of diagnoses as a means of reducing syndromal heterogeneity, and by doing so, enhance treatment response. Historically, the emergence of descriptive/categorical diagnoses in the early 1980s represented a step away from the vagaries of psychodynamic formulations. The progression from DSM-3 to DSM-5 improved clinical diagnosis by incorporating updated research-driven, descriptive/categorical criteria. In keeping with previous DSMs, there was less emphasis on the role of pathophysiology–etiopathogenesis in psychiatric diagnosis.

But there were shortcomings. Rather than defining discrete syndromes, the DSM approach ends up with complex disorders that overlap each other, share biomarkers and genetic traits across syndromes, and lack syndrome-to-effective treatment specificity. Psychopharmacologists also felt that high rates of co-occurring disorders and comorbidity impede precision medical approaches by contributing to polypharmacy, and low remission rates [31–33]. Neuroscientific researchers also needed to minimize heterogeneity [37–39].

In an era of evidenced-based medicine, better-designed randomized controlled trials reinforced concerns about factors related to incomplete treatment response, low remission rates, and treatment failures. These studies also reveal a curious paradox—many available psychotropics lack specificity for a distinct psychiatric disorder but are effective across spectrum of behavioral and psychiatric disorders. These data also suggest that we find ways to include etiopathogenesis, pathophysiology, and more specific endophenotypes in our diagnostic schemes. The National Institute of Mental Health (NIMH) proposed the RDoC/intermittent endophenotypes model as an alternative for research studies, but adapting this methodology to clinical settings is still a work in progress [37–39].

Many of these are not limited to researchers. Clinicians also grapple with a mix of patients. Some present with one diagnosis and a straightforward clinical response to treatment. Others are far more complicated, presenting with comorbid disorders, severe levels of residual functional impairment, and quickly exhausting published treatment

algorithms (assuming the clinician follows the algorithms and patients comply with treatment). These two subgroups lie along the extremes of a frequency/severity/service demands–distribution curve, suggesting clinicians need a multi-tiered approach to treatment.

One tier would involve non-patients with subsyndromal or diffuse patterns of discomfort, anxiety, misery, and unhappiness, but who do not seek pharmacotherapy. The second tier includes outpatients who fit DSM-5 criteria and respond to established treatment algorithms (current methods of psychotherapy and/or psychotropic drugs). These groups are best defined and treated with "lumping strategies" underlying most diagnostic schemas. The next two patient cohorts require multiple medication trials but finally settling in on a treatment regimen.

The last group is the significant minority of treatment-resistant patients with severe functional mental illness, multiple diagnoses and comorbidities, treatment resistance, vulnerability to relapse frequently during active treatment, or who experience ongoing symptomatic worsening. This population may require a "splitting" approach to fine tune their diagnosis using novel treatment precision pharmacogenetics, genome analysis, and diagnostic technologies to search for intermediate endophenotypes and pharmacogenomic subtypes [32–34].

Unfortunately, these dichotomies do not completely resolve things since diagnostic splitting (multiple subtypes of depression or schizophrenia) and lumping may not follow rules as originally advertised. The problem is that we are facing a group of complex polygenic, neurodevelopmental disorders that represent a combination of genetic variants with both small and large effect sizes, significant pleiotropy, and multiple epigenetic/gene–environment interactions. Many neuropsychiatric disorders also follow a neurodevelopmental trajectory that is affected by age of onset, severity, medical/neurological/neurodevelopmental comorbidities, diversity of clinical presentations, and limited treatment response [8, 20, 21].

Children with comorbid neurodevelopmental and behavioral/psychiatric disorders pose an additional set of problems—they sabotage easy classification models. For example, children with ASD and ID/IDD require more extensive, multidimensional assessments and a more systematic/ecological/educational intervention strategies that require closely monitored pharmacotherapy. Unfortunately, many children with co-occurring ID/IDD and ASD can be difficult to psychiatrically diagnose and treat. As a result, clinicians are treating a group of amorphous patients with a polypharmacy-based treatment plan.

These groups of high-risk children are difficult to split or lump and present the problem of trying to hit a moving target. Recently, Caspi and Moffitt (2018) described a "super lumper" strategy that takes a developmental perspective. They begin with internalizing–externalizing, generalized childhood–generalized emotional features that over time transform into subsyndromal diagnoses. In their construct, the progression toward "psychotic features" is the peak of severity for any psychiatric disorder. Their proposal creates a pyramid with severe mental disorder, treatment-resistant, psychotic individuals at the apex of a triangle [34].

Establishing the genetic risk for these severe cases can play out along many pathways. For example:

1. The child who expresses mania or depression or both during childhood may present with higher genetic loading for bipolar disorder (BD). The child's developmental trajectory and treatment with mood stabilizers can influence the course of the illness, but the outcomes remain variable. This group follows a homotypic course representing an early onset version of the parental disorder (bipolar parents = bipolar child), with similar patterns of treatment response.
2. A child at risk for bipolar disorder may present in early childhood with a range of impulsivity, anxiety, aggression, or other nonspecific symptoms. The child may or may not progress to classical bipolar disorder in adulthood but express recurring depression, anxiety, substance use, or a number of related psychiatric disorders. This group follows a heterotypic course that begins with generalized behavioral or psychiatric symptoms that evolve into a different adult disorder (anxious, behaviorally inhibited child who develops schizophrenia in adulthood), maintains a subsyndromal status, or never develops a primary psychiatric disorder [50].
3. There can be differences in adult outcomes and clinical presentation based on age at onset, greater risk for comorbidity, increased severity (including psychotic symptoms), and treatment resistance (including psychotic symptoms). These children may eventually require multiple treatments that land them in the "splitter" category for more specialized tertiary services [32, 34].

Each model assumes that there are significantly more nonspecific behavioral outcomes or less severe heterotypic disorders that might respond to generalized treatments. For polygenic disorders, this group may have lower gene loading, or lack other copy number variants or pleiotropic to develop the full syndrome or develop psychotic phenotypes. This early onset, full-syndrome cohort will develop a severe, high comorbidity, treatment refractory, and chronic mental illness. This subgroup of non-responders may need a splitter approach that requires a more detailed evaluation—including more precise genetic or neurobiological endophenotyping and new approach to psychopharmacology. Applying a pyramid model, this group would represent 10–20% of the population but require a much higher level of care, especially

since they tend to be resistant to many of our currently available psychotropic drugs [34, 42].

However, this lumper–splitter hybrid creates a dilemma.

1. Would it be wiser and more ethical to treat everyone as a potential non-responder and apply the "splitter" assessment and treatment protocols upfront? This super-splitter approach assumes that early, focused intervention or generalized therapies may prevent the progression to severe disorders in adulthood. For example, a majority of youth may not progress to schizophrenia but continue on with some level of functional impairment that affects their long-term well-being, educational achievement, and productivity. In an era of scarce resources, the project may be expensive, the yield might be low at first, and the gains (preventing a progression to end-stage disorder) may justify the upfront costs.
2. Would it be better to "wait and see" and focus scarce dollars on more intensive services for those who progress toward psychotic disorders, and more general services for those with minor symptoms?

Early intervention for children has the greatest chance for major changes in normalizing brain development and maturation. The question remains whether aggressive interventions prevent the onset of the illness, or improved adjustments prior to the onset of the full syndrome improve functional levels in adulthood. If we were to develop a generation of disease-altering drugs like mTOR inhibitors or mGlutamate-5 receptor antagonists, then using these early in development may provide a better chance than waiting until late childhood or adolescence (see Appendix 2). Today, much of our treatment focus is on symptomatic improvement, and remission is relatively uncommon. It is necessary that future psychopharmacological treatments move beyond symptomatic improvement but this will take some creative pharmacogenomic/pharmacogenetic innovations.

Multiple Choice Questions

1. Which of the following is the best example of drug induction of CYT P450 activity?
   a. Valproic acid increasing the serum drug level of carbamazepine
   b. Breakthrough menstrual flow on oral contraceptive after starting carbamazepine
   c. Loss of analgesic effects from codeine after starting fluoxetine
   d. Valproic acid toxicity with no significant changes in serum drug level after starting an OTC analgesic

   Answer: B. Increase hormone metabolism due to induction of CYT P450 3A4 by carbamazepine. Codeine is a prodrug that is activation by the CYT P450 2D6 pathway; adding fluoxetine inhibits this process. Valproic acid is highly protein bound and many drugs will compete and increase the free drug fraction with no change is serum drug level.
2. Pharmacokinetics encompasses which of the following processes?
   a. The mechanism of action of the drug
   b. The ability of the drug to directly influence gene activity
   c. The mechanism of action for drug allergies
   d. The excretion of drug metabolites

   Answer: D. The mechanism of action and gene activation are part of the pharmacodynamic package. Allergies are generally medicated by eosinophils and mast cell activity. Excretion of the metabolized drug, or in the case of lithium and dextroamphetamine where renal excretion predominates.

3. Many antipsychotic drugs block dopamine receptors. Which of the following is the best example of late occurring side effect of chronic antipsychotic treatment?
   a. Tardive dyskinesia
   b. Elevated prolactin levels
   c. Acute Dystonia
   d. Senile Dementia Alzheimer's Type.

   Answer: A. Elevated prolactin and acute dystonia occur early in the treatment course. There is no direct evidence that antipsychotic drugs cause dementia. TD is a late-onset movement disorder that is reversible in many patients. In children withdrawal emergent dyskinesias appear within the first month of drug withdrawal and resolve by 3 months after withdrawal.

4. Most antidepressants are extremely effective with nearly 100% of patients going into remission with their use.
   a. True
   b. False

   Answer: B. Unfortunately most psychotropic drugs produce a roughly 30% remission rate, and nearly as many do not respond to these treatments.

5. Pharmacodynamics deals with which of the following?
   a. Inhibition of 2D6 associated with a rise in fluoxetine blood levels
   b. The problem of ultra-rapid metabolizers and incomplete treatment response
   c. Drugs' effects on the PIP3 system and the regulation of intracellular calcium
   d. Excretion of lithium related to glomerular filtration rate

   Answer: C, A, B, and D are pharmacokinetic effects. The PIP3 system is connected to mood regulation and gene activation.

Appendix 1. Current Applications of Pharmacogenetics to the Psychiatry Treatment

At present (2018), there are 33 psychotropic agents commonly prescribed by psychiatrists with pharmacogenomic biomarkers in their FDA drug labels (Table 44.2).

(https://www.fda.gov/Drugs/ScienceResearch/ucm572698.htm) last accessed 10/24/2018, There are several notable warnings/contraindications against the use of certain medications with known genetic differences that can lead to lethal side effects, including the presence of human leukocyte antigen alleles HLA-B*1502 and HLA-A*3101 for carbamazepine and the closely related oxcarbazepine, and

Table 44.2 1A2, 2B6 2C9, 2C19, 2D6, 3A4, 3A5

Drug name	Type of warning	Relevant gene	Recommendations
Amitriptyline	Precautions	2D6	Caution in known 2D6 PM, or with administration with known 2D6 inhibitors
Aripiprazole	Dosage and administration; use in specific populations; clinical pharmacology	2D6, 3A4	For lauroxil form, dose changes are necessary with administration of 3A4 inhibitors and 2D6 inhibitors; for known 2D6 PM, dose change is different** (reduce dose to 441 mg from any higher dose if administering 3A4 inhibitor for longer than 2 weeks)
Atomoxetine	Dosage and administration; warnings and precautions; adverse reactions; drug interactions; clinical pharmacology	2D6	Dose adjustments may be necessary in concomitant use of strong 2D6 inhibitors; lower starting dose to 40 mg daily in adults who are known 2D6 PMs or concomitantly using 2D6 inhibitors
Brexpiprazole	Dosage and administration; use in specific populations; clinical pharmacology	2D6, 3A4	Administer half the usual dose with concomitant use of strong 2D6 or 3A4 inhibitors; for 2D6 PMs, administer half the usual dose; 2D6 PMs taking strong/moderate 3A4 inhibitors should receive one-fourth of usual dose
			For strong CYP3A4 inducers, double the usual dose
Carbamazepine	Black box warning; warnings; precautions	HLA-B*1502; HLA-A*3101; CYP3A4, 1A2, 2B6, 2C9, 2C19	Patients in genetically at-risk populations should be screened for the presence of HLA-B*1502 prior to starting treatment, and those with HLA-B*1502 should not be treated with carbamazepine unless risk clearly outweighs the precautions; the risks and benefits of treatment should be weighed before considering carbamazepine in patients known to be positive for HLA-A*3101; contraindication for concurrent use with nefazodone; caution for interactions with numerous drugs
Cariprazine	Clinical pharmacology	3A4	Dosage adjustments for concomitant use; halve current dosage if starting strong 3A4 inhibitor; not recommended to use concurrently with 3A4 inducers
Citalopram	Dosage and administration; warnings; clinical pharmacology	2C19; 2D6	The maximum recommended daily dose is 20 mg/day in 2C19 PM or with concomitant use of strong 2C19 inhibitors
Clomipramine	Precautions	2D6; 1A2	Concomitant use of drugs that can inhibit 2D6 may require lower doses than usually prescribed; it is desirable to monitor tricyclic antidepressant (TCA) plasma levels whenever a TCA is going to be administered with 2D9/1A2 inhibitor
Clozapine	Dosage and administration; use in specific populations; clinical pharmacology	1A2; 3A4; 2D6	For concomitant use of strong 1A2 inhibitors, use one-third of clozapine dose; for strong 3A4 inducers, concomitant use not recommended, however if necessary may need to increase clozapine dose; for moderate/weak 1A2, 2D6, and 3A4 inhibitors, monitor for adverse reactions; for moderate/weak 1A2/3A4 inducers, monitor for decreased effectiveness; for 2D6 PMs, it may be necessary to reduce clozapine dose
Desipramine	Precautions	2D6	Caution in known 2D6 PM, or with administration with known 2D6 inhibitors
Desvenlafaxine	Clinical pharmacology	2D6	May increase 2D6 substrate levels; reduce substrate dose to half if administering with 400 mg of desvenlafaxine
Diazepam	Clinical pharmacology	2C19; 3A4	2C19 PMs mentioned without recommendations in label, caution when administering with 2C19 or 3A4 inhibitors/inducers
Doxepin	Clinical pharmacology	2D6; 2C19	Caution when co-administering with 2D6, 2C19 inhibitors; half of maximum dose when co-administered with cimetidine

(continued)

Table 44.2 (continued)

Drug name	Type of warning	Relevant gene	Recommendations
Duloxetine	Drug interactions	1A2; 2D6	Avoid co-administration with 1A2 inhibitors; caution when administering with 2D6 inhibitors; contraindicated when co-administered with thioridazine
Escitalopram	Drug interactions; adverse reactions	2D6; 2C19	Caution when co-administering with 2D6 substrates; caution and monitor 2C19 PM's QTc; contraindicated in use with pimozide
Fluoxetine	Precautions; clinical pharmacology	2D6	A note on different composition of metabolites in 2D6 PM; caution with concurrent use with 2D6 substrates; contraindicated if concurrently taking pimozide and thioridazine
Fluvoxamine	Drug interactions	1A2; 2C9; 3A4; 2C19	Contraindicated in use with tizanidine (1A2 substrate), pimozide (3A4 substrate), ramelteon (1A2, 3A4, 2C19), and alosetron (1A2, 3A4, 2C9); caution when using with clozapine, methadone, mexiletine, theophylline, alprazolam, and diazepam
Iloperidone	Dosage and administration; warnings and precautions; drug interactions; clinical Pharmacology	2D6; 3A4	Dose should be reduced to half when administering to known 2D6 PM, or concurrently with strong 2D6 or strong 3A4 inhibitors

PM poor metabolizer, *RM* rapid metabolizer, *TCA* tricyclic antidepressant, *QTc* cardiac conduction: prolonged Q wave-T wave interval
References 3, 8–10, 26, 28, 51–58

known mitochondrial disorder caused by mutations in POLG for valproate. Most of these labels continue to pertain to those with known variants in the cytochrome P450 enzymes that leads to changes in its enzymatic activity, or with the concurrent administration of known inhibitors of certain CYP enzyme isoforms. Within the labels that pertain to CYP450 enzymes, variations in 2D6 and 3A4 predominate; however, there are also labels that pertain to 1A2, 2B6, 2C9, 2C19, and 3A5. Though most are warnings, some medications recommend dose adjustments if the patients have known differences in the enzymatic activity of certain CYP450 alleles [3, 51].

Those individuals that have copies of the genes that code for CYP450 enzymes that lead to poor metabolism of their enzymatic targets are referred to as poor metabolizers (PMs), and those with copies that lead to rapid or very rapid metabolism of their targets as rapid metabolizers (RMs) or ultrarapid metabolizers (UMs). There are numerous genetic changes associated with changes in enzymatic activity, including SNPs, deletions, translocations, and inversions that can affect both the coding and the noncoding areas surrounding the particular CYP450 isoform. There are also numerous genes that are not well studied but affecting enzyme function [3, 10, 28].

Limitations

1. The clinical application of defining neurobiological and intermediate endophenotypes is most helpful in providing more precise diagnosis (mininzing heterogeneity), matching mechanisms of drug action with the pathopysiology of the psychiatric disorders, and applying pharmacogenetics and pharmacokinetics to fine tune drug responsivity. This approach is most helpful when applied to patients with significant drug nonresponsiveness.
2. There are too many variations in the genome of unknown significance to guarantee that a person is not a PM or UM, or has a predisposition to potentially fatal side effects. The functional significance of the variations requires scientific investigation and follow-up after its discovery. The decision to study the frequency of a specific allele in a population depends on both its frequency and the availability of resources to conduct the follow-up studies.
3. How do clinicians interpret these pharmacogenomic findings? An overreliance on genotyping methods may uncover common variations in one population, but may have limited ability to catch rare but catastrophic variants in other populations. There is an overrepresentation of European samples or from those with European origin in pharmacogenomics studies, and a significant underrepresentation of those from Oceanic, Middle Eastern, South-Central Asian, and African heritage. This leads to disparities in both the significance and relevance of testing among different populations [51]. For example, the known genetically predicted prevalence of CYP2D6-poor metabolizers is significantly higher in those of European and Native American origin relative to other ethnic origins [52].
4. Pharmacogenomic differences based on ethnic origin have led to several illustrative examples with a significant impact on the delivery of psychiatric care. One is the recommendation that patients with genetically at-risk populations be screened for the presence of HLA*1502 prior to the initiation of carbamazepine, which is now considered standard of care in countries where the at-risk popu-

lation reflects a significant proportion of the population [53, 54]. Another illustrative example is the adoption of the benign ethnic neutropenia allowances in the administration of clozapine in African/African-Americans [55].

5. Being a normal metabolizer of an antidepressant, or having "adequate" serum levels, does not guarantee that the person would respond to said antidepressant. Perhaps the core issue is that measuring serum levels of SSRIs, Serotonin-norepinephrine uptake inhibitor (SNRIs), and antipsychotic drugs is not the standard of care or necessary. Many psychiatric professional organizations support systematic reviews on the topic to date, but have not recommended such testing prior to the initiation of antidepressants [28, 56].

Despite these concerns, many supportive recommendations by the Clinical Pharmacogenomics Implementation Consortium rely on this incomplete data. Many studies supported by industry support the clinical utility of these tests, including improved rates of remission/response to medication, and economic benefits with matching serum drug levels with clinical response is a way to o fine tune treatment and minimize the number of treatment nonresponders due to inaadequate dosing [57, 58]. Recent data challenge these reported advantages, and note that reduction in side effects or drug–drug interactions are their greatest strengths. These authors raise methodological concerns about the validity of the study data used to support these claims. But they are a first infant step toward precision medicine and time and more refined supporting data are needed [28].

Appendix 2. What New Treatment Ideas Are on the Horizon?

Each of these novel interventions expands our understanding of basic pathophysiology and neuropharmacology. Judging from the current literature, we may also need to continue our investigations due to the following:

1. Transcranial magnetic and direct current stimulations to key brain circuits are deepening our understanding of neuroplasticity and neuro-circuitry formation, and treatment of complex cognitive and emotional regulatory networks [59–64].
2. Ways to modify the effects of gut microbiome and neuronal connections to the brain via the vagus nerve [60, 65].
3. Expand and develop additional chronotherapies (treatment based on circadian rhythms) [59, 65].
4. Recognize and treat anti-neuronal antibody disorders that influence ion channels, second messenger systems, and intracellular molecular biology; role of antibodies (biologicals) directed to cell surface and intracellular antigens [64, 66, 67].
5. Modify dysregulated neuro-inflammatory and neuroendocrine pathways [3, 7, 74–78].
6. Complementary and Alternative Approaches: The use of antioxidants (*N*-acetyl cysteine, omega-3, folate derivative, inositol) for long-term efficacy studies; N-actetylcystiene (NAC) is helpful in some OCD-like and addiction behaviors; folic acid derivatives are used for treatment of refractory mood disorders; cannabinoids, oxytocin, and ketamine are perhaps the most encouraging ones for epilepsy, psychosis, chronic SCZ and ASD, and acute management of severe mood disorders [68–78].
7. Correct aberrant patterns of gene activation/regulation (epigenetic and intron/exon regulation of transcription factors; manipulating noncoding RNA effects on gene regulation, transcription, and translation) [79]
8. Modification of key enzyme systems related to metabotropic receptor regulation of local translation of proteins; mTOR inhibition; CRISPR9-CAS modifications of DNA for selected metabolic disorders; and stem cell implants [21, 24, 79].
9. Modification of mitochondrial function [22, 25].

Many of these newer interventions drill down to the basic pathophysiology of endophenotypes rather than treat specific symptoms or syndromes. Unfortunately, many have not trickled down into the world of children with neurodevelopmental disorders.

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Developmental Origins, Differential Susceptibility, and Resilience in Neurodevelopmental Disorders

45

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Learning Objectives

1. Understand the concepts of critical periods of development and sensitivity and developmental programming in the context of neurodevelopmental disorders.
2. Describe how specific prenatal environmental conditions, including maternal nutrition and stress, that, through interactions with one's genes, can contribute to offspring susceptibility to neurodevelopmental disorders.
3. Hypothesize about how an enriched postnatal environment provides an opportunity to overcome adversity experienced *in utero*.
4. Describe how the postnatal environment may worsen or mitigate the severity of neurodevelopment disorders, and how there are possible postnatal modulations of genetic predispositions.
5. Learn and understand how gene-by-environment interaction (GxE) models work and describe the different types of GxE models.

Highlights

- DOHaD and critical periods of sensitivity:
  - Early life environmental factors can profoundly influence an individual's health trajectory across their lifespan
  - The developing brain is especially vulnerable to environmental insults during the pre- and early postnatal periods, and adequate availability of nutritional factors and proper maternal hormonal signaling are critical for development to proceed undisrupted
- Prenatal exposures and susceptibility to ADHD:
  - Suboptimal maternal nutrition, metabolic state, or heightened stress during pregnancy may in part contribute to offspring susceptibility to ADHD, however, these exposures must be considered within the context of other factors (including genetics and the social environment) that contribute to a mother's health status during pregnancy
- Postnatal exposures and susceptibility to ADHD:
  - Toxicant exposure, nutritional factors, composition of the gut microbiome, sleep-wake cycle abnormalities, sleep apnea, relaxation, recreation, and the child's rearing environment may influence a child's biology and modulate gene expression, which has the potential to modify neurodevelopment, as in the case of ADHD
  - Epigenetic modifications are transient and interventions may alter the environment's effect on gene expression
- Modelling:
  - There are three types of GxE models: 1. diathesis stress, where specific genes place a vulnerable individual at increased risk of negative influence by adverse environments; 2. vantage sensitivity, where specific genes place susceptible individuals at increased likelihood of positive influences

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by enriched environments (delete the rest more likely increase positive influence by enriched environments on an individual); and 3. differential susceptibility, where specific genes increase influence (positive or negative) by the environment on an individual, for better or worse
- GxE models allow us to better understand how the environment influences each person differently, and this information can be used to devise better individualized treatment plans

Introduction

The Developmental Origins of Health and Disease (DOHaD) hypothesis, fostered by the seminal work of David Barker, posits that early life environments program developmental processes that determine later life disease risk and resiliency [1]. Where initially, research attended to the effect of maternal and fetal factors on adult organ function outside of the central nervous system, the importance of the prenatal period for the functioning of the brain and nervous system and psychiatric disorders has been confirmed [2–4] with evidence of the fetus as "a scientist in the womb," who collects, evaluates, collates, prunes, and consolidates neurosensory inputs in an ongoing effort to grow and change her own (body and) brain [5, 6]. Prenatal influences include toxicants, infections, nutrition, and obstetrical complications [7], as well as exposure to prenatal stress, which alone accounts for as much as 15% of the attributable risk for adverse mental health outcomes [8]. In this chapter, we focus on a common mental disorder, Attention Deficit Hyperactivity Disorder (ADHD), as an example of the influence of the environment and biological process across the lifespan for neurodevelopmental disorders (NDDs). We also report how one's susceptibility to the environment spans beyond the prenatal period and includes the influence of the social and emotional environment, hitherto less examined as significant influences. The work highlighted supports not only a developmental approach for understanding the emergence and modification of the trajectories of neurodevelopmental disorders, but also evidence of the continued susceptibility and resilience across the lifespan.

The Developmental Origins of Health and Disease

Consistent with the DOHaD hypothesis, the presence of appropriate stimuli and required resources, such as proper endocrine signaling or sufficient nutritive factors [9], can support the developing offspring to overcome the effects of some adversity, and constitutional factors such as one's genetic makeup can influence susceptibility to environmental insults or enrichment. Accordingly, considering both adversity *and* enrichment improves our ability to discern how early life circumstances program individual's health trajectories, through lasting changes to their metabolism, epigenome, and endocrine systems—a process also referred to as *developmental programming* [10].

Critical Periods of Sensitivity

During development, exposure to suboptimal environments can have a pronounced impact on the development of organs and biological systems, with lasting implications for disease risk or resiliency across the lifecourse. How adverse exposures experienced during sensitive periods of development affect offspring growth and development will vary depending on the *timing*, *type*, *duration*, and *magnitude* of the exposure [11]. During critical windows of susceptibility, a developing organ is especially sensitive to environmental influence as it undergoes rapid development [12]. These windows of vulnerability are organ-specific, as the rate or period of maturation will differ between and within biological systems [12]. The absence of appropriate stimuli during the correct developmental window can also be disruptive during periods of rapid growth and development, which occurs mainly in utero (*prenatally*) for humans, and during infancy and early childhood (*postnatally*).

Sensitive Periods of Brain Development

The developing brain is especially vulnerable to environmental insults during the pre- and early postnatal periods. The rapid birth, migration, and differentiation of neural cells that occur prenatally, as well as the maturation of neural cells and synapses and myelination that continues after birth [13], rely on adequate availability of nutritional factors [14] and proper maternal hormonal signaling [15] to proceed undisrupted. Further, while brain development occurs rapidly throughout the prenatal period and the first two years of life, it also continues through early adulthood [12]. Thus, exposures during childhood, adolescence, and early adulthood can also shape development that is occurring during these time periods and have a lasting impact on one's cognitive functioning and mental health [16, 17].

Early Life Environmental Factors That Influence Brain Development and Susceptibility to Neurodevelopmental Disorders

Within a DOHaD framework, we review how specific *prenatal* factors, including maternal nutrition, metabolic state, excessive exposure to stress, and mental health, as well as socioeconomic circumstances and genetics, can influence one's susceptibility to neurodevelopmental disorders, using Attention Deficit Hyperactivity Disorder (ADHD) in childhood and adolescence as a case example. Further, as the *postnatal* environment can be leveraged as an opportunity to improve outcomes in individuals with developmental susceptibility from genetics and the in utero environment, we will also highlight what is known about the contribution of postnatal environments to outcomes for individuals with ADHD.

Prenatal Environments

Prenatal Nutritional Environment

During pregnancy, a mother's diet and nutrient stores provide the nutritional support needed to maintain the rapid cell proliferation and tissue growth that accompanies fetal brain development. Macronutrients, minerals, and vitamins are critical for supporting the development and function of neural cells, synapses, and circuits [14], thus rendering the brain vulnerable to malnutrition during this critical period of development [11]. A better understanding of how the nutritional environment *in utero* may influence offspring susceptibility to ADHD is a key factor for understanding ADHD etiology, and for determining whether, and how, maternal preconception and antenatal nutrition can be optimized to improve neurodevelopmental outcomes for offspring.

Overall, maternal dietary patterns during pregnancy may influence a child's susceptibility to ADHD. Low maternal intake of fruits, vegetables, fish, and whole grains, or higher intake of processed foods throughout pregnancy, may associate with heightened ADHD symptomology in children aged three to eight years [18]. Additionally, prenatal maternal diets that are high in fats and sugars may associate with increased ADHD symptomology in youth (aged 7–13) via increased methylation of the insulin-like growth factor 2 (IGF-2) gene [19], which is believed to play a critical role in fetal neurodevelopment [20]. This finding illustrates that, in addition to their direct roles in cellular reactions, nutritional factors can influence neurodevelopment through epigenetic mechanisms.

Beyond the influence of overall dietary patterns, a number of specific micronutrients that are key for supporting proper neurodevelopment, including vitamin D, iodine, iron, and folates [14], are also important factors influencing ADHD susceptibility. Each of these micronutrients plays a direct and critical role in neurogenesis and neuron migration and differentiation [14], and when these essential nutrients are not available in sufficient supply, these developmental processes may be disrupted. A meta-analysis on the role of vitamin D in prenatal neurodevelopment reported that higher levels of 25-hydroxyvitamin D [25(OH)D], in either maternal blood during pregnancy or newborn blood at birth, are associated with reduced ADHD symptomology across the lifespan [21]. Further, insufficient maternal iodine intake at 22 weeks' gestation is associated with heightened ADHD symptoms at eight years of age in offspring [22]. Maternal iron-deficiency anemia diagnosed before 30 weeks' gestation, but not later in pregnancy (>30 weeks' gestation), is also associated with a higher risk of ADHD diagnosis in offspring aged 6–29 years compared to individuals born to mothers without anemia in pregnancy [23]. Notably, offspring susceptibility for ADHD decreased as gestational age at time of maternal diagnosis progressed, highlighting that the timing of in utero exposures is critical to understand their influence on neurodevelopment.

Despite the clear importance of folates to the developing fetal brain and central nervous system [24], relationships between perinatal maternal folate status and ADHD symptomology in children are less conclusive [25]. Some evidence reports no associations between periconceptional maternal folic acid (FA) supplementation and hyperactivity–inattention behaviors at age two [26, 27], while other research suggests FA supplementation may associate with a lower risk of hyperactivity and inattention symptoms in children at age seven [28]. Conversely, in children nine years of age, lower maternal red blood cell folate and dietary intake of folate in early pregnancy (14 weeks' gestation) are associated with higher hyperactivity and peer problem scores [29]. As the functional capacity of folate relies on additional nutrient cofactors, including vitamins B12, B6, and choline, considering the availability of these nutrients when investigating the relationship between maternal folate status and offspring susceptibilities to ADHD (currently rarely included) could help explain some of the contradictory findings. Lastly, evidence on whether maternal multivitamin supplementation during pregnancy may influence offspring ADHD susceptibility is mixed, with lower risk of ADHD and hyperkinetic–inattention disorders in children being reported in one study [28], but not in another [26].

Collectively, this research suggests that nutritional factors during pregnancy may in part contribute to offspring susceptibility to ADHD (Fig. 45.1); however, with prenatal nutrition should be considered other factors such as the child's genetic susceptibility and the social environment.

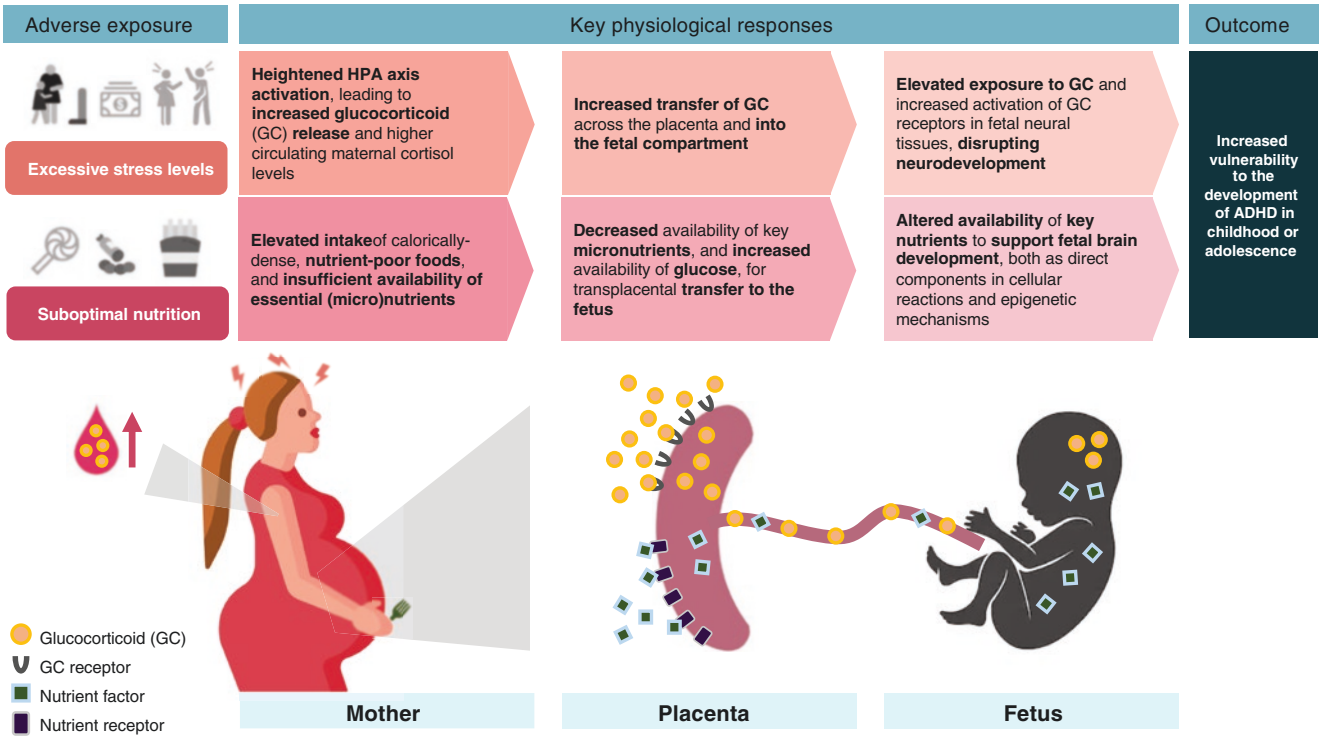

Fig. 45.1 Key mechanisms underlying the relationship between prenatal exposure to excessive maternal stress or a suboptimal nutritional environment and increased susceptibility to ADHD in offspring. Excessive stress during pregnancy can lead to heightened activation of the maternal hypothalamic-pituitary-adrenal (HPA) axis, releasing endogenous glucocorticoids (GC) into the maternal circulation. As a result, the fetus may be exposed to high levels of GC through transplacental transfer. As GC play an important role in normal developmental processes, the fetal brain expresses glucocorticoid receptors. In the event of heightened maternal HPA activation, these excess GC may bind to these receptors and disrupt typical developmental processes. A diet low in essential micronutrients may also have consequences for the developing fetal brain, as micronutrients play a direct and critical role in the formation and function of the brain and central nervous system. When excess fats and sugars are made available via diet, transplacental transfer of glucose may be increased. Together, the altered availability of these key nutrients (where in excess or insufficiency) can be disruptive for fetal neurodevelopment

Maternal Metabolic State and Hypertensive Disorders in Pregnancy

Evidence is emerging that an altered maternal metabolic state during pregnancy can increase a child's susceptibility to developing ADHD in childhood or adolescence. Type I diabetes in the mother is associated with an increased risk of ADHD in offspring [30] and higher rates of ADHD medication use in adolescents 13–19 years of age [31], and gestational diabetes mellitus is associated with increased likelihood of ADHD diagnosis in offspring aged 4–11 years [32]. Although the mechanisms linking *in utero* exposure to maternal diabetes and ADHD are not well understood, research in animal models suggests that epigenetic changes and oxidative stress in the mother and fetus, driven by high maternal glucose levels, may have detrimental effects on developing fetal neural tissues [33].

Further, the results from three meta-analyses suggest that in comparison to children born to mothers with a normal pre-pregnancy body mass index (BMI; 18.5–24.9 kg/m^2), children born to mothers classified as overweight before pregnancy (BMI 25–29.9 kg/m^2) may be more susceptible to developing ADHD by as much as 30% [34–36], and children whose mothers have obesity (BMI ≥ 30 kg/m^2) may be more susceptible by 60% [35]. Notably, excess weight gain during pregnancy is also associated with increased likelihood of subclinical ADHD presentation in children aged 4–11 years [32]. Epigenetic modifications, dysregulated leptin signaling, and increased levels of circulating pro-inflammatory cytokines as a result of chronic inflammation are posited to associate with maternal overweight and obesity, and may therefore be consequential for the developing fetal brain [37].

Maternal hypertensive disorders in pregnancy may also influence a child's susceptibility to developing ADHD. A systematic review and meta-analysis reported that in utero exposure to maternal hypertensive disorders may increase a child's likelihood of developing ADHD by 30% in comparison to children whose mothers did not have a hypertensive disorder during pregnancy [38]. Exposure to preeclampsia specifically, which is a placental-associated hypertensive disorder, may increase risk of ADHD in offspring by 15% (in comparison to children not exposed to preeclampsia) [39].

Hypertensive disorders in pregnancy associate with poor placental perfusion and dysfunction [40], which, in turn, may adversely impact the developing fetal brain through hypoxia and a reduction in nutrient availability. In all, the evidence suggests that suboptimal maternal metabolic states or hypertensive disorders during pregnancy are critical to consider when identifying infants who may be susceptible to developing a neurodevelopmental disorder and may benefit from early intervention.

Maternal Mood, Anxiety and Adverse Life Events

Maternal exposure to psychological stress during pregnancy, including prenatal depression, pregnancy-related anxiety, trauma or bereavement, war, and natural disaster, may also contribute to offspring risk of ADHD [41–43]. Here, we review this body of literature and summarize possible mechanisms through which this maternal stress may impact fetal brain development and influence susceptibility to neurodevelopmental disorders like ADHD.

Prenatal Depression

Depression is one of the most common complications in pregnancy, with up to 10% of pregnant people receiving a diagnosis for depression, and up to 20% of those with depression in pregnancy experiencing significant symptoms [44]. A few studies demonstrated a strong relationship between maternal depression during the prenatal period and ADHD symptomology in children aged three to six years [45, 46]. For example, in two large cohorts (Generation-R and ALSPAC), greater prenatal maternal depression and anxiety were both predictive of child attention problems at three to four years of age [45].

Prenatal Worries and Anxiety

While anxiety and depression have overlapping symptoms [47] and social risk factors [48], prenatal maternal anxiety is characterized by distinct symptoms of worries and concerns about pregnancy [47]. Similar to maternal depression, maternal anxiety during pregnancy is associated with childhood attention and hyperactivity symptoms [49, 50], and is predictive of an ADHD diagnosis in children aged 4–15 years [51–53]. For example, in an intra-familial study of 376 children with ADHD and their unaffected siblings (aged 6–12), mothers were more likely to report that they had experienced higher stress severity during the pregnancy with their affected child than the pregnancies of their unaffected siblings [54]. One study found that the impact of exposure to prenatal maternal anxiety may be most pronounced for restlessness and impulsivity behaviors [55].

Adverse Life Events

Prenatal adverse life events also associate with the development of ADHD in children [56–58]. These events include problems with pregnancy, loss of a close friend or relative, or problems with children, partners, or money [59]. Adverse life events are associated separately with inattention symptoms (e.g., attention shifting, but not focusing) in 4-year-old children [47] or with hyperactivity up to 16 years of age [57]. In those whose mother's experienced adverse life events prenatally, the risk of ADHD is possibly worse for those exposed during the second trimester and when the mother has lower social support and higher avoidance coping strategies [56]. Bereavement, as a separate stressor, has also been associated with later ADHD [60, 61] and ADHD medication use in childhood [62]. There is little evidence for the association of ADHD with natural disasters or human-made disasters (e.g., war).

Prenatal Stress and Genetic Susceptibility

ADHD is highly heritable, with recent heritability estimates from a meta-analysis of ADHD studies and recent twin studies calculated to be higher than 70% [63, 64]. A number of candidate genes have been identified through large-scale, genome-wide association studies (GWAS; Table 45.1). Furthermore, using 20,000 individuals with ADHD and 35,000 controls, the Psychiatric Genomics Consortium (PGC) developed a polygenic risk score (PRS) for ADHD, by aggregating significant genetic loci associated with ADHD into one factor [65–67]. Research on direct genetic effects for ADHD has been enriched by reports that individual genetic susceptibility also interacts with prenatal stress to predict child developmental outcomes [68].

Single gene variants known to be associated with ADHD have been studied in interaction with prenatal stress in Gene-by-Environment interaction (G×E) designs. For example, the 7-repeat allele of the Dopamine Receptor D4 (DRD4) gene variable number tandem repeat (VNTR) polymorphism [63, 69] interacts with prenatal stress to associate with ADHD. Carriers of the DRD4 7-repeat have an increased risk of developing ADHD when exposed to prenatal stress; however, in the absence of prenatal stress, these carriers displayed the lowest symptomology of all children [54]. These contrasting outcomes suggest joint effects of genetic risk and environmental exposures, like prenatal maternal stress, on offspring ADHD risk. Similarly, the *COMT* polymorphism (rs4680), which is associated with cortical thickness and frontal cortical surface area in ADHD [70], interacts with prenatal maternal anxiety to predict ADHD symptoms in individuals 3–15 years old [51]. There appears to be no report on the joint effect of the ADHD PRS and prenatal stress yet.

An interesting contribution to knowledge on the role of heritability in the association between exposure to prenatal stress and ADHD comes from studies with birthing mothers who are either biologically related or unrelated (having conceived through *in vitro* fertilization with donor gametes) to their child. In one such "cross-fostering design," which

Table 45.1 Examples of genes with known roles in the pathogenesis of Attention deficit hyperactivity disorder (ADHD) pathogenesis of ADHD

Gene	Name	Gene role	Characterization of gene in ADHD
DAT1 or *SLC6A3*	Dopamine transporter	Transports dopamine into the cell	DAT1 prefrontal cortex (PFC) expression-based gene network moderates the impact of perinatal conditions known to increase the risk for ADHD on executive function in childhood [89]
DRD2	Dopamine receptor D2	G-protein-coupled receptor that inhibits adenylyl cyclase activity	The "A" allele carriers have been shown to relate to lower ADHD symptoms compared to G allele carriers of DRD2 [174]
DRD4	Dopamine receptor D4	G-protein-coupled receptor that inhibits adenylyl cyclase activity	The 7-repeat (7r or long) allele of a VNTR polymorphism in exon III of DRD4 is a validated risk allele for ADHD [63, 69]
FTO	FTO alpha-ketoglutarate-dependent dioxygenase	Role in nervous and cardiovascular systems and a strong association with body mass index, obesity risk, and type 2 diabetes	The FTO rs8050136 SNP has been associated with ADHD diagnosis [175]
GRIN2B	Glutamate ionotropic receptor NMDA type subunit 2B	Brain development	GRIN2B rs2268119 SNP and lower SES shown to associate with attention problems [176]
LPHN3 or *ADGRL3*	Adhesion G-protein-coupled receptor L3	Cell adhesion and signal transduction	LPHN3 rs1868790 and rs6551665 SNPs have been shown to associate with ADHD diagnosis [177]
MAOA	Monoamine oxidase A	Neurotransmitter breakdown	Low activity of the MAOA genotype and low birth weight are associated with more ADHD symptoms [174]
MTHFR	Methylenetetrahydrofolate reductase	Processes amino acid/folate metabolism	Maternal C677C MTHFR CT gene polymorphisms are significantly higher in individuals with ADHD compared to the TT and CC alleles [178]
SLC6A4	Solute carrier family 6 member 4	Transport of serotonin from synaptic spaces into presynaptic neurons	Higher SLC6A4 promoter methylation status has been shown to be significantly associated with worse clinical ADHD symptoms [179]
ST3GAL3	ST3 beta-galactoside alpha-2,3-sialyltransferase 3	Catalyzes the transfer of sialic acid from CMP-sialic acid to galactose-containing substrates	Increased expression of ST3GAL3 has been significantly associated with ADHD [180]

Information on gene roles provided from Genetics Home Reference (181)

examined ADHD as a function of genetic susceptibility from the donor and the prenatal environment [71], prenatal stress associated with offspring ADHD symptoms in biologically related mother–child dyads only. This suggests that the effect of prenatal stress on ADHD is in part heritable.

Mechanism

The mechanisms underlying prenatal stress inform our understanding of the pathways leading to adverse neurodevelopmental outcomes and mental health trajectories in offspring who are exposed to maternal stress prenatally. One important target has been the Hypothalamic–Pituitary–Adrenal (HPA) axis, the body's stress regulation system, with the hypothesis that heightened maternal stress during pregnancy associates with dysregulation of the HPA axis and an increase in circulating glucocorticoids in the mother, which, through transplacental transfer, leads to increased fetal exposure to endogenous glucocorticoids [72]. As the HPA axis plays a critical role in embryonic development and maturation of organs, including the brain [73], elevated exposure to glucocorticoids, or exposure during the wrong time of development, it has the potential to adversely affect fetal neurodevelopment [74] (Fig. 45.1). One rare study examining prenatal maternal stress, ADHD, and the stress response finds that intimate partner violence (IPV) during pregnancy is associated with ADHD symptoms in children [75]. However, the child's salivary cortisol secretion profile after a stressful challenge (the high secretion profile) only predicted internalizing symptoms, not ADHD. These results point to lasting consequences of pregnancy IPV on ADHD susceptibility in children, but the effect of a child's cortisol regulation on ADHD is less clear [75]. Notably, the evidence linking prenatal maternal stress to adverse child neurological outcomes through dysregulation of maternal stress hormones has not been consistent in human studies [76].

Epigenetic changes have also been examined as an underlying molecular mechanism linking prenatal stress and adverse developmental outcomes [77, 78]. Associations between cortisol-related placental mRNA expression and temperament during the first two years of life have been reported [79], and although there are no similar findings for ADHD to date, there is good evidence that individuals with ADHD have different patterns in gene polymorphism, in parental gene transmission, methylation, DNA composition, and RNA expression [80, 81]. Some gene variants highly associated with ADHD (e.g., *DAT1* or *SLC6A3*) are highly susceptible to epigenetic modifications [82], and others (e.g., DRD4 and *5-HTT* regions) have higher levels of methylation

in the umbilical cord tissue of children who later develop ADHD symptoms [83].

Other pathways through which prenatal stress influences neurodevelopment, including through the child's immune response [84] and the microbiome [85], are important to consider in the development of a unified model of prenatal stress. However, to date, there is limited evidence for linking these pathways to NDD risk in human populations.

Sex-Specific Responses

Consistent difference in the prevalence of ADHD by sex has led to explorations of sex-specific vulnerability to ADHD [86]. For example, sex-specific vulnerability of the dopaminergic system, 5-HT1A receptor binding potential, HPA axis activity, and stress vulnerability and responsivity could explain the sex-specific differences in outcomes like ADHD [56]. Sandman et al. proposed that exposure of male fetuses to adversity increases their risk of serious neurodevelopmental outcomes, while female fetuses adapt to in utero stress, with a trade-off of vulnerability to fear/anxiety and impaired executive functions later in development [86]. In addition to investigating the interaction between genetic susceptibilities and environmental exposure, it appears important to further characterize sex- and gender-specific mechanisms to understand the etiology of ADHD and other NDD.

Summary

Taken together, prenatal environmental factors, such as the mother's nutrient status, metabolic state, exposure and response to stress, and mental health and well-being, are consistent and critical determinants of a child's neurodevelopment and their susceptibility to neurodevelopmental disorders, specifically ADHD symptoms, diagnosis, and cognition. The role of the stress regulation system, genetic susceptibility, and epigenetic changes is better characterized to date than the role of the microbiome, immune response, and findings in neuroimaging. Key research direction could also include stress regulation pathways, and bioinformatics approaches to strengthen the identification of genetic susceptibility from specific gene network or expression profiles of brain regions specific to ADHD [87–89]. Intervention could be informed by the identification of pathways associated with ADHD that are modifiable postnatally. As it stands, there already exist important opportunities to shape neurodevelopment after birth, given the important pace of brain development in the first two years of life and even into early adulthood [12].

Postnatal Modifiers of Prenatal Programming

Environmental insults during the postnatal period have the potential to amplify the effects of prenatal adversity on development [90]. Conversely, environmental enrichment during the postnatal period presents an opportunity to mitigate the consequences of adverse prenatal exposures and correct developmental trajectories. The study of postnatal gene-by-environment interaction is in its infancy, yet will provide promising insights into how postnatal environmental factors may moderate genetic predisposition to neurodevelopmental disorders. Here, we discuss postnatal environmental factors that may help shape neurodevelopmental trajectories, but explicitly leave out mention of psychotherapeutic and psychopharmacological interventions, which are comprehensively covered elsewhere (Chap. 44) in this textbook. These postnatal exposures could be clinically relevant and guide pediatric and public health practices, as well as an integration of home, school, health, and urban planning.

Toxicants, Nutrition, and the Microbiome

Postnatal toxicant exposure, breastfeeding practices, probiotic use, and nutrition can modulate neurodevelopment. In the context of ADHD, it is known that exposure to air pollution [91], arsenic [92], and bisphenol A (BPA) [93] during the postnatal period increases risk of ADHD. Prenatal exposure to BPA is reported to exert changes in the expression of several genes involved in the pathophysiology of ADHD in two separate animal models [94]. Further, genetic susceptibility and pollutant exposure in the prenatal period may, through epigenetic mechanisms, determine *how* vulnerable one may be to the health effects of pollutant exposure later in life [95].

There is also a role for postnatal nutrition and a number of key micronutrients in one's susceptibility to ADHD, as children and adolescents with iron-deficiency anemia [96] and vitamin D deficiency [97] may be more susceptible to developing ADHD. As ADHD is associated with dysregulated dopaminergic signaling [98] and iron is a cofactor for the synthesis of dopamine [99], it is possible that insufficient iron intake could contribute to ADHD symptomology through dopaminergic dysregulation [100]. Furthermore, vitamin D is a critical component for the synthesis of serotonin [101], which plays a critical role in social behavior, executive function, and impulsivity [102]. Thus, vitamin D deficiency may alter activity in the serotonin pathway and contribute to ADHD symptomology [102]. Lastly, ADHD has been associated with shorter periods of exclusive breastfeeding (<6 months), or shorter duration of any breastfeeding, in children and teens aged 7–17 with ADHD, in comparison to their counterparts without ADHD [103]. Together, this suggests that optimizing the early postnatal nutritional environment may be a key target for reducing one's risk of ADHD later in development.

The gut microbiome is also being increasingly recognized as a key postnatal contributor to neurodevelopment. Although the literature is only emerging for NDDs, there is some evi-

dence for a link between gut microbes or beneficial bacteria and brain function and structure. In a randomized controlled trial investigating the use of probiotic *Lactobacillus rhamnosus* GG given to infants for the first six months of life, children receiving the probiotic were significantly less likely to be diagnosed with Autism Spectrum Disorder or ADHD at age 13 (0/40) compared to the placebo group (6/35), even after controlling for child gender [104], indicating that the microbiome may play a role in the disorders. Further, there are differences in the composition of bacterial taxa between young adults with ADHD and adults without. Those with ADHD have an increase in *Bifidobacterium* genus [105]. In teenagers, *Bacteroidaceae* is elevated in those with ADHD; moreover, both *Neisseriaceae* and *Neisseria spp*. are potential biomarkers of ADHD [106]. Ten-year-old children with ADHD have a different gut composition than controls and more concurrent constipation and flatulence [107]. Germ-free mice humanized with fecal microbiota from ADHD patients have decreased white matter structural integrity of the hippocampus and corpus callosum, with reduced functional connectivity between motor and visual regions of the brain [108], a finding supporting the microbiome–gut–brain paradigm. Thus, nutrient sufficiency, toxicity, probiotics, and the impact of the microbiome during *both* the pre- and postnatal periods may be modifiable factors important not only in our understanding of the development of ADHD, but also in the mitigation and lessening of symptoms.

Sleep Hygiene

There are other modifiable exposures that are associated with higher risk for ADHD, including poor sleep pattern or hygiene and obstructive sleep apnea–hypopnea syndrome (OSAHS). Sleep disturbances are commonly observed in children with ADHD. Interestingly, there are several genetic polymorphisms near the *CLOCK* gene, an important regulator of the sleep–wake cycle, which are associated with ADHD and could explain sleep problems in patients with ADHD [109]. Furthermore, *DAT1* and *COMT* polymorphisms, already introduced, are differentially associated with sleep–wake cycles in young adults [110]. Although preliminary, a 30-min morning 10,000-lux bright light therapy beginning 3 h after mid-sleep time significantly advanced the sleep phases in adults with ADHD. The phase advances in dim light melatonin onset and mid-sleep time were correlated with lower ADHD symptoms and hyperactivity–impulsivity sub-scores [111].

In addition to a genetic predisposition to sleeping problems, adenoid hypertrophy is more severe in individuals with comorbid ADHD than in those with OSAHS alone [112]. While the etiology of adenoid hypertrophy in ADHD remains to be clarified, OSAHS is commonly caused by tonsil and adenoid hypertrophy that result in the complete or partial collapse of the upper airway, causing sleep and ventilation disturbances. Hypoxia or sleep disturbances from OSAHS may be a risk factor for the development of ADHD, and thus early surgical intervention on tonsil or adenoid hypertrophy could be considered as a strategy to prevent ADHD. For example, adenotonsillectomy significantly reduced problematic behavior in children aged 3–12 [113] and 6–11 years [114].

Mind and Body

There is also emerging evidence that relaxation and recreation, through yoga or mindfulness practices or play and exercise, may also improve ADHD symptomology. While there is some evidence that mindfulness practices may benefit individuals with ADHD [115, 116], a review that reports positive effects from yoga and mindfulness meditation for youth with ADHD warns against these methods as first line interventions due to methodological limitations and low rigor of evidence [117]. A recent review of physical activity interventions on cognition and behavior in young people with ADHD finds that acute exercise improves cognitive function, including processing speed, memory, and executive functions [118]. However, the effects of the intervention on behavior or the underlying biological mechanisms (e.g., epigenetic changes or cortisol activity) [119–122] remain unclear [118]. Finally, access to and play in outdoor green or blue spaces may also have a positive impact on ADHD symptoms [123, 124].

Rearing Environment

One of the most widely studied and recognized postnatal exposures associated with neurodevelopmental disorders, including ADHD, are Adverse Childhood Experiences (ACEs), which encompass a wide range of psychological, physical, and socioeconomic stressors during childhood. ACEs have been shown to associate with worse ADHD clinical symptomology [125–130] and increased risk of an ADHD diagnosis [131–133]. Although trauma clearly accentuates neurodevelopment, understanding the role of normal variants of parenting and the postnatal rearing environment provides key evidence about more prevalent parental behaviors and experiences.

There is emerging evidence on mother–child interactions and neurodevelopment suggesting that parental mental health [71], sensitivity, and warmth may be protective against NDD, while poor maternal care may be detrimental [134–137]. For example, more sensitive and less intrusive parenting improved inhibitory control and mitigated the ADHD symptoms in five-year-olds who manifested anger reactivity at four months of age [135].

Adoption and IVF studies have also examined the role of the rearing environment for ADHD, in view of addressing methodological concerns that parenting behaviors do not themselves lead to ADHD, but rather they reflect the genetic vulnerability for ADHD in the parent, which is transmitted genetically to the offspring (gene–environment correlation). In the "cross-fostering" study described previously, greater

postnatal maternal anxiety or depression increased children's ADHD symptoms at six years, separately from biological relatedness [71]. In the Early Growth and Development Study (EGDS; $N = 345$), ADHD symptoms in offspring were examined in relation to biological, surrogate, and adoptive ADHD symptomatology and rearing environment. In a first study, they report that overparenting and adoptive parent's internalizing symptoms can lead to attention problems [138], separately from the biological risk for ADHD transmitted from the donor. In a follow-up design, they unpacked some of the mechanistic pathways underlying the child's risk of ADHD by mapping how the parental hostility and interparental conflict, which in part lead to greater symptoms of ADHD in the child, and evoked by the susceptible child in their dyad with their parent [136, 139, 140]. Given that ADHD in children is associated with higher parental stress and lower self-efficacy and marital satisfaction than parents of children without ADHD [137], these studies of postnatal parenting exposure imply that parents of children who are genetically susceptible to ADHD may benefit from child-rearing support and moderate later ADHD severity [139]. This would be consistent with evidence that the association between adverse childhood events and psychopathology is in part mediated by changes in gene expression [141].

Modeling the Joint Effect of Genetic Susceptibility and Environmental Exposures in Developmental Programming

In light of the reviewed evidence about the interplay of the early environment and genetic susceptibility, we present in this section approaches and models for exploring models of gene-by-environment interactions. Using a specific example of the joint effect of maternal depressive symptoms and child genetic susceptibility in the prediction of a child's Negative Emotionality (NE), we model how to approximate the strength of influence and the direction of the influence (beneficial or adverse) an environment may have on the developmental programming of an individual.

The Three Types of Gene-by-Environment Interactions Models

There are many ways to conceptualize how an individual's response to their environment is shaped by their genetics. These models form the basis of gene-by-environment interactions (G×E); they stipulate that some individuals are *susceptible* to factors in their environment that can adversely alter development and health trajectories, while others, *resilient* individuals, are not (or very weakly) affected by these same environmental factors. The first model, called *diathesis-stress*, states that certain *vulnerable* individuals are disproportionately negatively affected by negative environments [142]. This corresponds to the classic model in psychiatry where every behavioral or mental pattern is labeled as healthy or disordered, and genotypes may confer higher vulnerability ("risk"), but never advantage. The second model, called *differential susceptibility*, states that certain individuals may be *susceptible* to both positive and negative environments, for better or worse [143–145], thus implying that genetic susceptibility is not necessarily a net-negative, but may be beneficial under positive/enriching environments. Of note, differential susceptibility is similar to the concept of *biological sensitivity to context*, by Boyce and Ellis [146], which provides evidence that individuals are *developmentally plastic* rather than only vulnerable [147]. Finally, the third model, called *vantage sensitivity*, states that *vantage sensitive* individuals may be positively influenced by positive environments, while unaffected by negative environments [148].

These three types of models (diathesis-stress, differential susceptibility, and vantage sensitivity) can be further differentiated by whether they are *weak* or *strong*. *Strong* models assume that the resilient individuals, those unaffected by the adverse environment, are fully resilient, with no impact whatsoever of the environment. *Weak* models tend to reflect more common observations, namely that individuals resilient to adverse environments manifest some adverse outcome, but much less so than that manifested by susceptible individuals [149, 150].

It is important to note that the type of G×E model depends on factors in environment and the outcome. For example, a child's attention may be impaired by high levels of maternal depression (diathesis-stress), but their negative affectivity may be improved by low levels of chaos, or impaired by high levels of chaos (differential susceptibility). Understanding what type of interaction occurs for each phenotype and environment is very important, as they might differ and inform different interventions. Specifically, understanding which type of G×E best explains the outcome, could help identify those who are very susceptible to adverse environments (diathesis-stress) and would fare better with positive changes, and those for example who might excel in enriched environments (differential susceptibility or vantage sensitivity). We next define the G×E model and its components, demonstrate how to fit such a model, and show how to determine the type of G×E interaction.

Definition of a G×E Model

A G×E model for a gene g, environment e, and continuous outcome y, can be represented as:

$$y = \beta_0 + \beta_e e + \beta_g g + \beta_{ge} ge + \varepsilon,$$

where β_0, β_e, β_g, and β_{ge} are respectively the intercept, environment main effect, genetic main effect, and gene-by-environment interaction effect, and ε is the unexplained error. If the outcome is categorical (e.g., Major Depressive Disorder: Yes or No), then one must apply a transformation (using the sigmoid function) on the linear model to obtain a prediction on the probability of obtaining each category.

Components of the G×E Model

Environment

As discussed throughout this chapter, perinatal environmental factors help to shape an individual's developmental and health trajectories. When we include environmental variables in a risk model we may, for example, consider maternal depression score (continuous), prenatal diabetes (binary), or prenatal nutritional deficiencies (categorical).

Genetics

There are many types of genetic variations but the most common is the single nucleotide polymorphism (SNP), which is a single variation in a nucleotide (A, C, G, T or U) at a specific location in the genome. Since humans are mostly diploid, we can usually observe three nucleotide variants at a location in the genome. For example, for an SNP with alleles C and T, there are three possible genotypes, CC, CT, and TT. In practice, we can assign a genotype as being associated with "susceptibility" (or risk, or vantage sensitivity) and dichotomize accordingly (e.g., CC or TT is risk, while CT is resilient). Alternatively, we can consider continuous risk, and note genetic "dose" accordingly (e.g., CC = 0 [no risk], CT = 0.5 [some risk], TT = 1 [high risk]). We generally ignore SNPs for which there is little human-to-human variation (rare SNPs with low allele frequency, e.g., less than 5%, or usually present SNPs) as this means that we cannot reliably determine the influence of having or not having this SNP. Furthermore, SNPs in linkage disequilibrium (LD) are generally omitted from consideration in G×E, *viz.*, non-random association of alleles at several sites.

In a G×E model, we may consider one or multiple genetic factors. Since a single genetic variant is equivalent to a needle in a haystack, we generally want to consider more than one genetic factor per model. We often do so through *genetic scores*, which are weighted sums of multiple genetic factors. Genetic scores have many names in the literature, but their meaning remains the same; they can be called: "multilocus genetic profile" [151, 152], "allelic score" [153, 154], "SNP score" [155], "genotype score" [156], "genetic prediction score" [157], and most commonly "polygenic risk score" [158–161]. The weights of the genetic scores (how each genetic factor is weighed) are most often determined by effect sizes estimated from independent (discovery) genome-wide association studies (GWAS) [162]. They can also be directly estimated within a model by using the Latent Environmental & Genetic InTeraction (LEGIT) approach [163, 164].

How to Test for the Type of Interaction?

There are two main approaches to determine the type of an interaction in the G×E model: (1) Regions of Significance (RoS), and (2) Confirmatory and Competitive Models (CCMs).

The first approach is the classic method by Aiken, West, and Reno [165]. This approach determines at which values of the environment is the effect of the genetic variable (we call this the simple slope) significantly different from zero. We classify the interaction as:

(a) Diathesis-stress, when the simple slope is significant only at low environmental quality (Fig. 45.2a).
(b) Differential susceptibility, when the simple slope is significant at both low and high environmental qualities (Fig. 45.2b).
(c) Vantage sensitivity, when the simple slope is significant only at high environmental quality (Fig. 45.2c).

This can be visualized in Fig. 45.2. The Regions of Significance (RoS) approach depends entirely on whether we obtain significant *p*-values in only low, only high, or in both low and high levels of environmental quality; this is very problematic in small samples as *p*-values are notoriously unstable and sensitive to small sample sizes [166]. Small samples lack the power to detect small effects, which means that the rate of false-negative (non-significant effect even if there is a real effect) can be high. This suggests that RoS can be unreliable in small samples. In fact, RoS has been shown to have poor performance in practice, even with a sample size as big as $N = 1000$ [163].

Confirmatory and Competitive Models (CCMs) form a modern approach to determining the type of G×E interaction. This approach consists of fitting models representing each type of interaction and choosing the model with the best fit (using any goodness-of-fit criterion such as the Akaike information criterion [AIC] and the Bayesian information criterion [BIC]) [167, 168]. In CCMs, we must first re-parametrize the G×E model as

$$y = \beta_0 + \beta_e (e - c) + \beta_{ge} g (e - c) + \varepsilon,$$

where c is the cross-over point between the lines for different levels of genetic susceptibility. As shown in Fig. 45.2a–c, the cross-over point is at $c = min\ (e)$ for vantage sensitivity, at $c = max\ (e)$ for diathesis-stress, and anywhere in the range

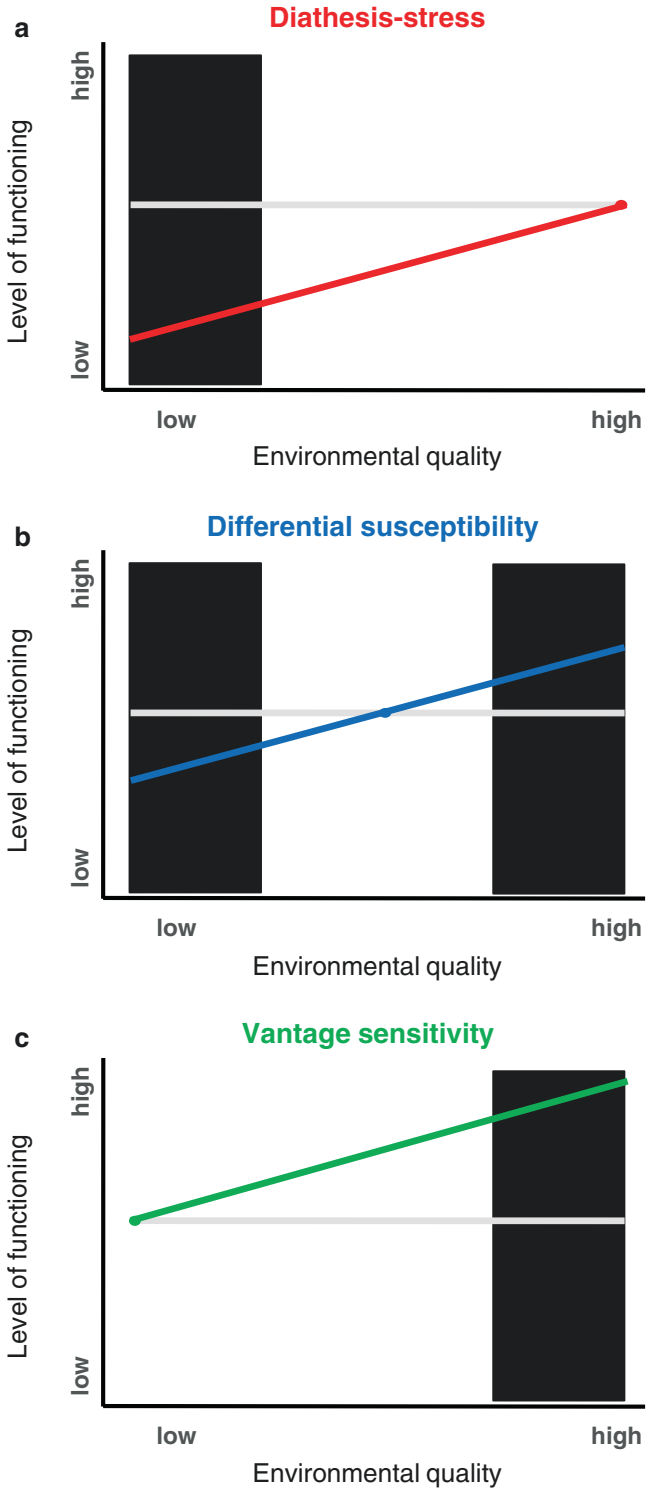

Fig. 45.2 Regions of Significance GxE models for (**a**) diathesis-stress, (**b**) differential susceptibility, and (**c**) vantage sensitivity interactions. From "Distinguishing differential susceptibility, diathesis-stress, and vantage sensitivity: Beyond the single gene and environment model" https://psyarxiv.com/27uw8 under CC0 1.0 license (https://creativecommons.org/publicdomain/zero/1.0/)

$[min(e), max(e)]$ for differential susceptibility. To allow a bit more flexibility, CCMs also separate the three types of interactions into weak and strong models. A strong model assumes that resilient individuals are imperious to the adverse environment (therefore the slope $\beta_e = 0$), while a weak model assumes that resilient individuals manifest some (modest) adverse outcome from the adverse environment (therefore $\beta_e \neq 0$). Thus, CCMs fit one model per type of interaction (based on the re-parametrization equation):

1. Weak vantage sensitivity (β_e estimated, $c = min\ (e)$)
2. Strong vantage sensitivity ($\beta_e = 0$, $c = min\ (e)$

Weak differential susceptibility (β_e estimated, c 45.estimated)

3. Strong differential susceptibility ($\beta_e = 0$, c 45.estimated)
4. Weak diathesis-stress (β_e estimated, $c = max\ (e)$)
5. Strong diathesis-stress ($\beta_e = 0$, $c = max\ (e)$)

The interaction is represented in Fig. 45.3. We then assess the fit of each model, which allows us to determine which of the six models is most likely (i.e., best fit). However, if the estimate of c has a 95% confidence interval that falls beyond the observable range of the environment (i.e., not in $[min(e), max(e)]$), one should reject models 3 and 4 even if they have slightly better fit than other models.

Modeling with Examples

Determining the type of interaction using RoS and CCMs can be done through the respective functions G×E_interaction_RoS and G×E_interaction_test from the LEGIT package in R [163, 164]. For detailed examples on how to fit such models with LEGIT, please read the vignette "G×E Testing" and the reference manual (all of which can be found at https://cran.r-project.org/web/packages/LEGIT/).

This section illustrates G×E models for the prediction of Negative Emotionality [164, 169], a measure of sadness, fear, and aloneness in negative situations. Prenatal maternal mood is known to be associated with NE in offspring [170, 171]; higher levels of maternal depression increase the risk of the child NE, while lower levels of maternal depression decrease the risk of child NE. Yet not all children have the same susceptibility to maternal depression; some children could even be resilient and thus unaffected by maternal depression.

In the original paper by Green, Babineau et al. [169], they found a significant interaction between prenatal maternal depression and a genetic variant in the serotonin receptors

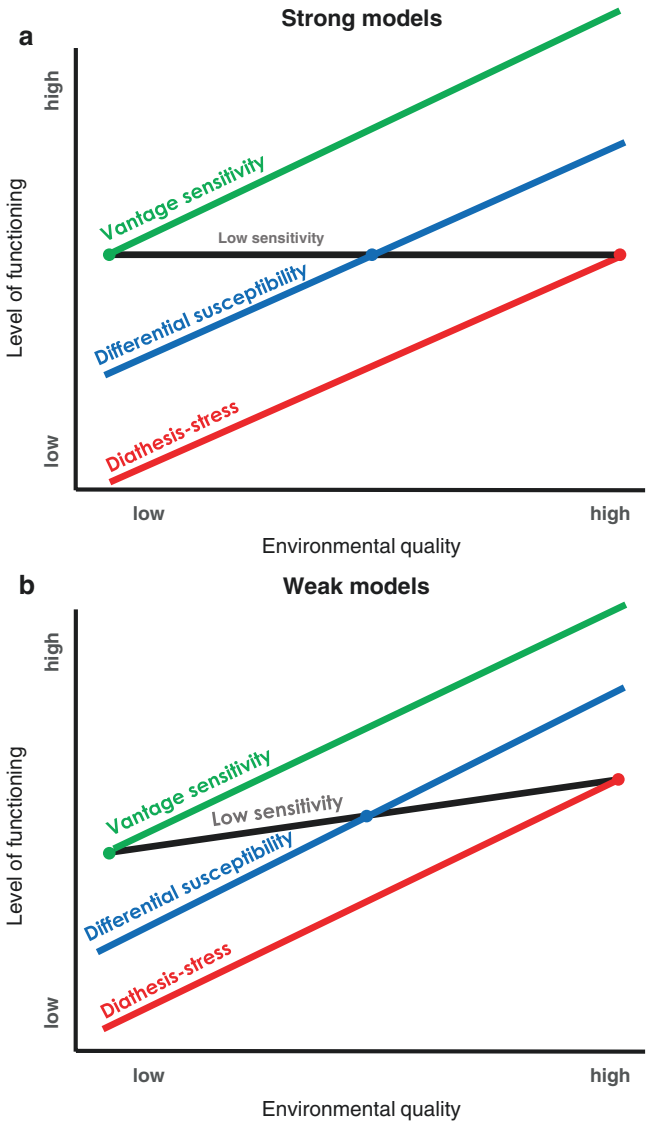

Fig. 45.3 Confirmatory and Competitive Models GxE models. Visual representations of (**a**) strong models and (**b**) weak models. From "Distinguishing differential susceptibility, diathesis-stress, and vantage sensitivity: Beyond the single gene and environment model" https://psyarxiv.com/27uw8 under CC0 1.0 license (https://creativecommons.org/publicdomain/zero/1.0/)

Table 45.2 The prediction of negative emotionality (NE) at 3, 6, 18, and 36 months of age from the interaction of prenatal maternal depression and a multilocus genetic score

Predictors	G × E model
G × E	
Intercept at 3 and 6 months (IBQ)	0.41*
Intercept at 18 and 36 months (ECBQ)	0.23
Prenatal depression	−0.01
Genetic score	0.76***
Prenatal depression × genetic score	0.05**
Genetic score	
DRD4 (6, 7, or 8 repeats)	0.19^{t}
5-HTTLPR (S/lg)	0.44***
OXT (AC or AA)	0.37***
Covariates (inside G × E)	
Postnatal depression (at latest previous time point)	0.01***
College education	0.16*
Material/social deprivation index (quintile)	−0.06**
Mother's age of birth	−0.02*

$^{t}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

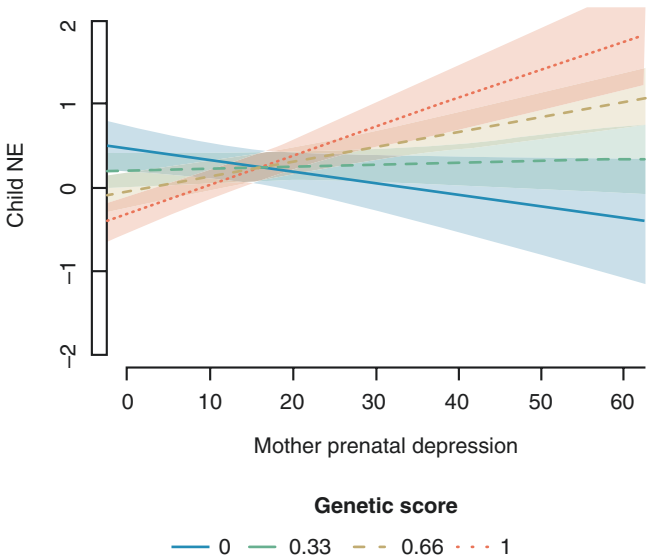

Fig. 45.4 The interaction effect of prenatal maternal depression and a genetic score (of 5-HTTLPR, DRD4, OXT) on offspring negative emotionality (NE) at 3, 6, 18 and 36 months. A higher genetic score in the offspring is associated with increased early negative emotionality when the mother experiences more depressive symptoms throughout pregnancy

called 5-HTTLPR predicting child NE early in life. Later, Jolicoeur-Martineau, Wazana et al. [164] found a significant interaction between maternal depression and a genetic score, consisting of three genetic variants, to predict child NE early in life. The three genetic variants, 5-HTTLPR, DRD4, and OXT [172, 173], correspond respectively to genes in the serotonin receptors (linked to mood), dopaminergic receptors (linked to attention), and oxytocin receptors (linked to bonding). The G×E model with the three genetic variants is represented in Table 45.2 and Fig. 45.4.

From Fig. 45.4, we see that those with low genetic score are resilient, while those with high genetic score become more susceptible to maternal depression (e.g., more depression = more NE, and less depression = less NE). In Table 45.2, we highlighted the critical information. We see that maternal depression does not have a significant effect; this means that maternal depression does not affect the child's NE when the genetic score is zero (which corresponds to low genetic susceptibility). In other words, resilient children have a genetic score of zero, and they are unaffected by maternal depression. We see significant positive effects for genetic score and the interaction between depression and genetic score; this means that a higher genetic score leads to more NE and more susceptibility to maternal depression (through the interaction

effect). In LEGIT, where the weights of the genetic score sum to 1, we see that DRD4 contributes positively to 19% of the score (trend association), 5-HTTLPR contributes positively to 44% of the score (significant association), and OXT contributes positively to 37% of the score (significant association).

The last step in G×E modeling is determining the type of interaction (diathesis-stress, differential susceptibility, or vantage sensitivity). The G×E model with three genetic variants was not tested, but the original G×E model with only 5-HTTLPR showed evidence for either diathesis-stress or differential susceptibility. In Fig. 45.4, we see that the crossover point (where the lines cross) is around 16. For the measure of depression used in this model, mothers with a depression score lower than 16 are considered non-depressed. In contrast, mothers with a depression score of 16 or above are considered clinically depressed. Thus, if we assume diathesis-stress, we can interpret the model as saying that if mothers are clinically depressed, children with a high genetic score have worse NE, in which case the three genes involved would all be considered harmful (they cause no harm if the environment is positive or cause harm if the environment is negative). Meanwhile, if we assume differential susceptibility, we can interpret the model as saying that (1) if mothers are clinically depressed, children with high genetic score have worse NE, and (2) if mothers are non-depressed, children with high genetic score have better NE, in which case the three genes involved would be considered either a blessing (if living in a positive environment) or a curse (if living in a negative environment). Accordingly, by fitting a G×E model and testing the type of interaction, we learn about the influence of specific environments and about individual susceptibility.

Conclusions

Specific environmental exposures experienced during critical periods of development, including nutritional insufficiency, altered maternal metabolic states or hypertensive disorders, or excessive maternal stress and mental health challenges, can make a child more susceptible to developing ADHD (Fig. 45.5). However, exposure to these adverse prenatal environments is not deterministic. The brain remains

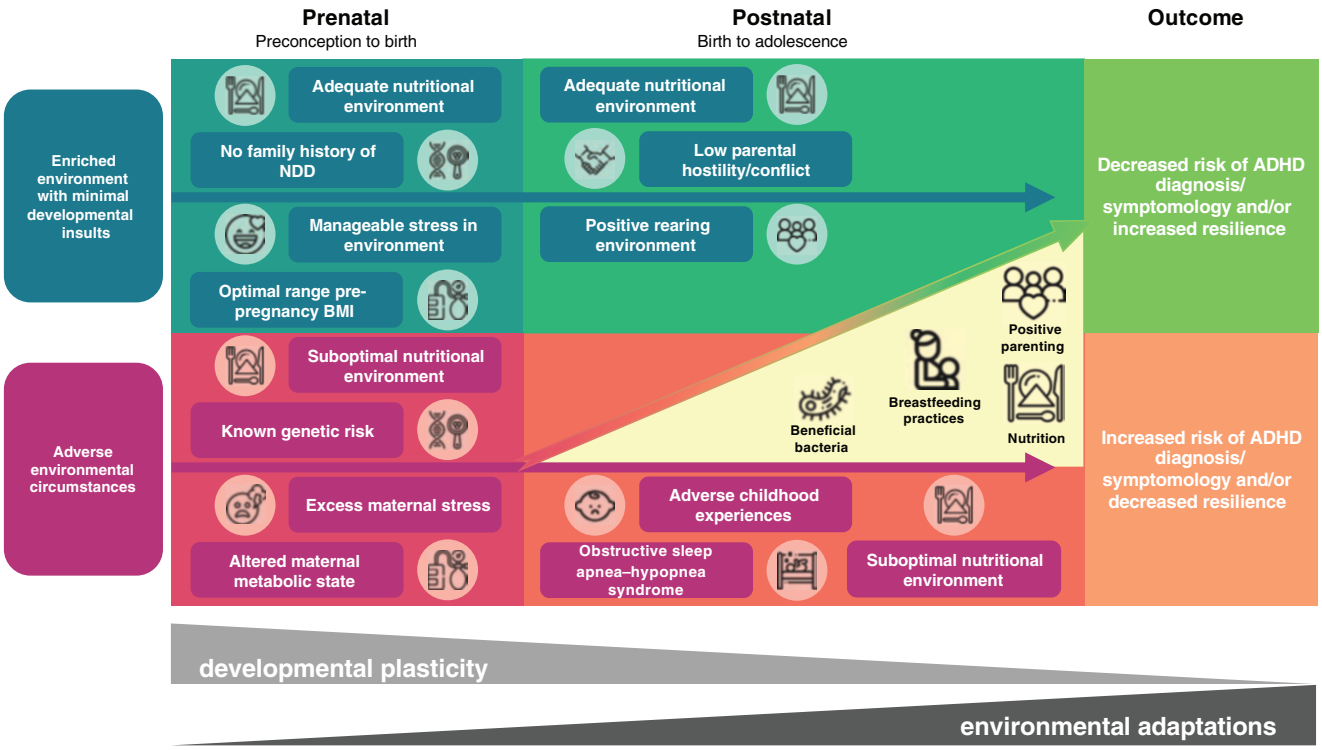

Fig. 45.5 Enriched and adverse perinatal environmental factors influencing a child's susceptibility to ADHD. Prenatal environmental factors including nutrition, genetics, maternal stress and metabolic state influence the development and symptomology of ADHD in children and adolescents. Maternal nutrition during the perinatal period consisting of adequate micronutrients (such as iodine, iron, folate and vitamin D) and fewer processed foods may decrease the risk of ADHD diagnosis or symptomology in children. Prenatal maternal stress, as well as an altered metabolic state during pregnancy, has also been shown to play a role in the development and symptom severity of the disorder. Genetics also play a critical role one's susceptibility to developing ADHD, as illustrated by the strong heritability for the disorder and the identification of genes that contribute to the pathogenesis of ADHD (Summarized Table 45.2). In the postnatal environment, adverse childhood experiences, insufficient micronutrients (including iron and vitamin D) and obstructive sleep apnea–hypopnea syndrome (OSAHS) have been associated with increased risk of developing ADHD. Modifiable factors that may play a protective role in increasing resilience include supplementation with probiotics that contain beneficial bacteria, breastfeeding practises, parenting style and adequate nutrition

plastic in the postnatal period and fully matures in early adulthood [12]. As such, there is a possibility to curb neurodevelopmental disorders or their severity through specific and timely interventions. For example, interventions to address maternal nutrient status, metabolic health, stress regulation, and mental health during the periconceptional and prenatal periods may help to reduce the occurrence of adverse exposures for the fetus and have positive effects for in utero neurodevelopment. Further, support for interparental conflict, parent–child hostility and parental mood, improved sleep quality and quantity, more exercise and stress relieving activities, as well as nutritional support, during the postnatal period, serve as opportunities to remedy developmental trajectories set in utero.

Multiple Choice Questions

1. Does vantage sensitivity imply:
   A. A bad outcome with a negative environment and a good outcome with a positive environment
   B. A normal/average outcome with a negative environment and a good outcome with a positive environment
   C. A bad outcome with a negative environment and a normal/average outcome positive environment
   Answer: B
2. In children with ADHD who were exposed to _____ , ADHD symptom severity and symptoms were more pronounced in children with a DRD4 7R polymorphism.
   A. Maternal smoking during pregnancy
   B. Green spaces
   C. Prenatal maternal stress
   D. Sleep therapy
   Answer: C
3. The Developmental Origins of Health and Disease (DOHaD) hypothesis states that:
   A. The external environments one is exposed to across their lifespan contribute equally to their disease risk and resiliency.
   B. Maternal health during pregnancy programs developmental processes in their offspring, conclusively dictating later life disease risk and resiliency.
   C. Early life environments, including periconceptional, prenatal and early postnatal factors, program developmental processes that determine later life disease risk and resiliency.
   D. Maternal health during the periconceptional period is the greatest determinant of offspring disease risk and resiliency across the lifespan.
   Answer: C
4. The following statement describes the relationships between prenatal maternal stress and mental health, and offspring risk susceptibility to ADHD:
   A. ADHD diagnosis or symptom severity is increased in offspring born to mothers who experienced mental illness, including depression, bereavement or traumatic stressors during pregnancy, but not in cases of prenatal maternal anxiety.
   B. ADHD in the child has been strongly linked with maternal trichotillomania in response to psychological stressors, including bereavement, war, natural disaster, trauma, and pregnancy-related anxiety.
   C. Prenatal paternal stress has a greater contribution to offspring ADHD susceptibility than maternal stress during pregnancy.
   D. The impact of maternal stress during pregnancy on ADHD symptomatology may be most pronounced for internalization, externalization, emotional liability, restlessness and impulsivity behaviours, while having no influence on intelligence quotient.
   Answer: D
5. Which postnatal factors have been shown to have a possible protective effect against the development of ADHD?
   A. Probiotic supplementation for first 6 months of life, adequate breastfeeding practices and sufficient intake of iron and vitamin D.
   B. Daily exercise, vitamin B12 supplementation and a positive rearing environment.
   C. Sufficient water intake, low parental aggression and vaginal delivery.
   D. High dietary protein, vitamin C and supportive parenting style.
   Answer: A

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Part VI

Neurodevelopmental Disorders in the Context of Families

Children with Neurodevelopmental Disabilities and the Right to Health

46

Keiko Shikako, Jonathan K. Y. Lai, Shikha Saxena, and Maya Sabatello

Learning Objectives

- Understand the right to health in international law in the context of childhood disabilities.
- Consider the research evidence on rights-based approaches for children with disabilities.
- Appreciate the possible applications of rights-based approaches in childhood disability health research and services.

Highlights

1. Two human rights frameworks—the CRC and the CRPD—support the rights of children with disabilities. These universal rights can support and guide health services, policies, and research.
2. Children with disabilities are, for multiple reasons, at higher risk of not having their right to health met in different situations, for instance, in regard to their reproductive health as they are vulnerable to sexual abuse and poor reproductive and sexual education, in emergency responses to disasters when accessibility options are not considered, and in health promotion initiatives that are not inclusive of different needs.
3. Countries that are signatory of the CRC and CRPD should pursue and develop policies aimed at disseminating information and increasing public awareness of the Conventions into health and social policy. Nationwide education campaigns are recommended to sensitize the population at large (UN Committee on the Rights of the Child 1995, Section D-19), and the use of rights-language in official government documentation.
4. Potential solutions, based on multidisciplinary research evidence, to promote the human rights of children include: 1) Promoting youth and parent agency: awareness and engagement, 2) Providing individual supports and reasonable accommodation across services, 3) Promoting active citizenship by universal design in all areas of healthcare, 4) Adopting person- and family-centered approaches, and 5) Building capacity, awareness, and coordination of services using a rights-based approach.
5. Youth and parent agency has been highlighted as fundamental to promote human rights. Youth with disabilities and their families are often not aware of their rights, are not informed about and do not partake in decision-making processes related to them. These are contrary to the conventions' notion of agency and representation. Actionable items in this area can be incorporated into health services and research, and included recommendations for parents to use a supportive parenting approach to teach the child self-agency (Berrey and Nielsen 2007). Similarly, health care providers should facilitate child agency during consultations, medical and rehabilitation procedures and services. For communities and community programs, increasing the offer of culturally relevant services for families of those living with disabilities of different ethnic backgrounds was also suggested as a practical direction to improve youth and parent agency.

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An Introduction to Human Rights and Rights-Based Approaches in Childhood Disabilities

The Convention on the Rights of the Child (CRC) was adopted in 1989, and entered into force in 1990, with the goal of creating a new world for children [1]. It was the first hard-law instrument to acknowledge children as subjects of the law; the first comprehensive document to address the myriad of challenges in promoting the well-being of children in a variety of settings; and importantly, the first international, legally binding instrument to require States to acknowledge children's agency, evolving capacity, and voices in decisions relating to them. As the implementation of the CRC is progressing worldwide (even if slower than originally hoped), there is a growing recognition of the importance of adopting rights-based approaches (RBAs) for promoting children's well-being in different levels of decision-making, programming, and services.

A Human Rights based approach can be defined as "a conceptual framework that is normatively based on international human rights standards and operationally directed to promoting and protecting human rights. It seeks to analyze obligations, inequalities and vulnerabilities, and to tackle discriminatory practices and unjust distributions of power that impede and undercut human rights" [2]. Aside from the humane, value-laden system that such an approach entails, a rights-based approach has been recognized as a prime way for citizens to claim their rights and to hold their States responsible for acts of omission or commission. Thus, for example, while a State's adoption of a policy that contradicts its obligations under international law in relation to the right to health may be held responsible for acts of commission, a State's failure to take appropriate steps toward the realization of children's right to physical and mental health would count as an act of omission [3].

Notwithstanding the promotion of rights-based approaches by international bodies such as the United Nations (UN), and the World Health Organization (WHO), and incremental adoption and implementation of such approaches by governments worldwide, the rights of children with disabilities have lagged behind. It is likely that the CRC's limited reference to this special group of children (only one article, Article 23, refers to this group) along with its content, which delineated the discussion on children with disabilities to States' discretionary provision of only certain services rather than full inclusion in society, has played a role in this neglect [4].

The Convention on the Rights of People with Disabilities (hereinafter: the CRPD; adopted in 2006; entered into force in [5]) is the newest international human treaty and as such has adopted a participatory approach on its development, including persons with disabilities on all stages, and aimed at addressing the neglected needs of persons with disabilities in society. In particular, the CRPD provides the most comprehensive document to date to address the rights and needs of people with disabilities, including children. Although it seemingly only reiterates rights that were included in the CRC, the CRPD paves the way to include children with disabilities in the conversation by promoting accessibility in all spheres of life, ensuring provisions to highlight children (and other groups who face multiple vulnerabilities like women) in the reporting and monitoring mechanisms, and facilitating a twin-track approach that both mainstreams disability throughout States' children's rights obligations and singles out unique interests and needs of children with disabilities to the implementation of the rights of children with disabilities [6].

Human Rights Application in Signatory Countries

A key component of international law is that States that are signatories to the Human Rights Conventions should abide by international processes of reporting and monitoring. The reporting process is in place to ascertain that countries will implement rights-based approaches as part of their policies and programs, and will make concrete efforts in all areas of governance to, incrementally, realize the human rights of the populations covered by these human rights treaties. Both the CRC and the CRPD have been widely adopted in the international community. The CRC, in particular, has been ratified by all countries in the world, except the United States [7], whereas the CRPD has been ratified by 181 countries [8]. In Canada, for instance, the CRC has been ratified in 1991 [9] and the CRPD in 2010 [10]. Thus, as an example of a country that has been a signatory to several human rights treaties, the government of Canada, as a State party to the convention, has the obligation to report regularly to the UN on advances in implementation and monitoring of the convention [11].

While the implementation and monitoring process are held between governments and the UN Office for the High Commissioner on Human Rights, the CRPD also creates several opportunities for the disability community to participate in the monitoring process and contribute to advancing human rights issues locally. Governments have to produce a report to the UN stating how they have advanced the implementation of the conventions in that given period of time, including population-based data that prove that advances have been made in the different articles, and that rights-based approaches framework is being adopted and advanced at all levels of government. At the same time, researchers, civil society organizations, health care providers, educators, and the community at large have opportunities for producing parallel reports, presenting community-based data, administrative data, and lived experiences, and any other information that may provide a realistic picture of how human rights are being realized on the ground. This can be done in tandem

with the government, but also in parallel over a reporting cycle. Different stakeholders can support producing, for instance, a List of Issues Prior to Reporting (LOIPR) [12, 13]. This LOIPR should describe examples that demonstrate the extent to which the points presented by government are being represented in the daily lives of children with disabilities (Across the CRC/CRPD), and for each of the articles in the given convention.

The Right to Health and Its Intersections with Health Frameworks

Adopting a human rights framework to health may not be intuitive in most fields of health research and services. Frequently associated to the social sciences and humanities fields, a human rights approach to health is often used exclusively in the context of litigation, and negative rights—that is, when health is lacking—but not in a positive rights approach: when health should be promoted and granted for the simple reason of being a human [14]. In an old conception of health as the absence of disease [15], the notion of health and disability was also dichotomized: either you had health, or you had a disability [16]. This conceptualization has gradually changed in the last decades with the introduction of new visions of health such as the International Classification of Functioning, Disability, and Health (ICF) [17]. The international push to adopt a right to health framework to health is recent and is slowly being integrated into cultural and political developments to influence the way health, treatment, functioning, and disability are conceptualized [18–20]. The ICF provides perspectives on how socioeconomic status (SES), environmental conditions such as where people live, and the availability of resources and services can influence their health [21]. In addition, the WHO also provides detailed information on the underlying factors, or social determinants of health [22], to help better promote the health outcomes of people with disabilities by addressing inequalities and ensuring the consolidation of their human rights.

The right to health is articulated in the CRC (Article 24) as recognition that children must enjoy the highest attainable standard of health and access to facilities for the treatment of illness and rehabilitation of health. "States Parties recognize the right of the child to the enjoyment of the highest attainable standard of health and to facilities for the treatment of illness and rehabilitation of health. States Parties shall strive to ensure that no child is deprived of his or her right of access to such health care services" (CRC, Article 24). The CRC recognizes that children with disabilities may require additional health and rehabilitation services, and should have access to those services, with a focus on achieving full integration, and development in all areas including cultural and spiritual development (CRC, Article 23). The CRPD recognizes the right to health through equal access to mainstream and specific services within general health services, inclusive health insurance, and free and informed consent (CRPD, Article 25). Indicators for the CRPD monitoring are operationalized through structures (e.g., health legislation that is equitable, laws and regulations that give woman and girls with disabilities access to sexual and reproductive health and education, and national accessibility standards for health), processes (e.g., proportion of public health services that are inclusive and trained to receive persons with disabilities, complaints related to disabilities in the health care system), and outcomes (e.g., birth and death registration of persons with disabilities compared to other persons, prevalence of undernourishment or malnutrition among children with disabilities) [23]. The CRPD also describes specific recommendations related to rehabilitation and habilitation (Article 26), including a cross-sectoral rehabilitation systems and services for all people with disabilities with no discrimination and availability, knowledge, and use of assistive devices and technologies for persons with disabilities.

These articles and procedural indicators establish an excellent base for benchmarking health service provision for children with disabilities and their families, elucidate mechanisms of action, and anticipate potential benefits of having positive rights-based approaches put in places through policies and systems. They also provide a comprehensive list for health researchers to consider questions that can help creating these indicators, and support families in advocating for the rights of their children with governments and services.

The Right to Health and Childhood Disability

Individual and social impact of disability is diverse and far reaching. Years Lived with Disabilities (YLDs) have been reported to increase from 537.6 million in 1990 to 764.8 million in 2013 with musculoskeletal, mental, neurological, substance use disorders, and chronic respiratory diseases being the key drivers of this increase [24]. Recent research also shows consistently that severity of childhood disability is strongly correlated with socioeconomic status [25], which is further aggravated by the limited access to health care, education, and other supports crucial for their well-being [26]. The multiple layers of vulnerabilities—disability, low SES, and poor access to care—could be addressed through the adoption of a human rights framework lenses.

The promotion of the right to health for children with disabilities requires the participation of all levels of health services to include public health, primary to tertiary health care, and community sectors. In fact, timely access to a well-coordinated system of care is fundamental to enable people with disabilities to achieve their full potential, both person-

ally and socially [18]. Despite years of research advocating interaction between public health and disability, people with disabilities, and particularly children, are often not included in research and public health practices and initiatives [27]. Children with disabilities are, for multiple reasons, at higher risk of not having their right to health met in different situations, for instance, in regard to their reproductive health as they are vulnerable to sexual abuse and poor reproductive and sexual education, in emergency responses to disasters when accessibility options are not considered, and in health promotion initiatives that consider only able-body children [28]. These issues point to the need of thinking more practically about integrating human rights and social determinants of health lenses at academic, clinical, community, and government levels [27].

Lack of understanding of how these elements come together as risk factors for children with disabilities not only negates the right to health, but also feeds into a society that is constantly oblivious of the potential deleterious effects of not taking positive measures to implement, monitor, and measure the use of rights-based frameworks. This omission may deprive generations of children and families of the supports that are crucial for their health and well-being [29].

The right to health for children with disabilities is mentioned under different forms, both in the CRC and CRPD. The rights enshrined in the CRC are categorized by the following four guiding principles: non-discrimination; best interest of the child; the right to life, survival, and development; and the right to participate. However, while providing a dedicated and comprehensive framework of the rights of children, the CRC does not address the significant barriers that children with disabilities experience in realizing those rights. The CRPD, in contrast, demands a fundamentally different approach to disability, centered on the social model of disability and the notion of inclusion with the objective of creating a fully inclusive environment for persons with disabilities, including children. The right to health is therefore understood in a perspective of different elements of inclusion in society: primary and tertiary health services, health promotion initiatives, health education, and healthy environments. Essentially, the recommendation is to create opportunities for children and youth with disabilities to be considered in any initiative, policy, and program, including, obviously, health research and clinical practice [28].

Rights-Based Approaches in Childhood Disabilities: What Is the Research Evidence?

A recent scoping review identified multidisciplinary research evidence on the value of rights-based approaches to promote the health of children with disabilities and identified actionable solutions ([30] submitted). This review included studies spanning from medical research and law, to social sciences, policy studies, and social services and identified several themes related to the rights of children with disabilities, including: assistive technology, client-centered approaches, multi-agency care, financial or social assistance from the State, integration and inclusion, family-centered care, full citizenship, measuring successful outcomes, employment, reasonable accommodation, youth engagement and agency, access to information for families, education programs for families to advocate, universal design, improving specific services, and lifespan approach to care. Barriers to implementation of rights-based approaches were also identified and included: limitations in community participation, regional discrepancies, legal recourse, structural disadvantages, resource challenges, stigma, family stress and caregiver burden, and transitioning from child to adult services.

More importantly, the review captured potential solutions, based on multidisciplinary research evidence, to promote the human rights of children, and they were grouped into five themes: (1) Youth and parent agency: awareness and engagement; (2) Individual supports and reasonable accommodation; (3) Promoting active citizenship by universal design; (4) Person- and family-centered approaches; and (5) Service provider capacity building and coordination.

The themes of youth and parent agency were particularly important across studies, acknowledging that youth with disabilities and families of children with disabilities are often not aware of their rights, and are not informed about and do not partake in decision-making processes related to them. These are contrary to the conventions' notion of agency and representation, where the principle of participation is key and integral part of the general obligations. Actionable items in this area can be incorporated into health services and research, and included recommendations for parents to use a supportive parenting approach to teach the child self-agency [31]. Similarly, health care providers should facilitate child agency during consultations, and medical and rehabilitation procedures and services. For communities and community programs, increasing the offer of culturally relevant services for families of those living with disabilities of different ethnic backgrounds was also suggested as a practical direction to improve youth and parent agency. For governments, the CRC and CRPD committees recommend that countries pursue and develop policies aimed at disseminating information and increasing public awareness of the Conventions. Nationwide education campaigns are recommended to sensitize the population at large (UN Committee on the Rights of the Child 1995, Section D-19), and the use of rights-language in official government documentation. By increasing awareness about the Conventions, individuals with disabilities, their families, and communities will be equipped with information that can facilitate action and advocacy efforts promoting rights-based approaches.

Individual supports and reasonable accommodations are an integral part of the Support programs that meet the individual needs of people with disabilities, and are an essential component of equality principles that are primordial for the implementation of human rights [32]. Traditionally, support programs are understood under the lenses of "reasonable accommodations"—mechanisms or mitigation strategies to address barriers and needs based on a diagnosis or medical condition. This approach is largely based on the medical model of disability, rather than the social model of disability, and reflects a "negative rights" approach to human rights implementation. Under a social model of disability paradigm, the environment or the social, political, and contextual structures have a key role in providing "reasonable accommodations" by default [33]. Social and contextual structures that follow this approach provide community spaces that go beyond non-discrimination practices to ensure affirmative equality—that is, community spaces that accommodate the needs of all individuals, regardless of their disabilities, and allow for equal participation in the activities, and equitable use of all spaces [34].

The CRC enshrines the right to community integration and the right to family life, mandating States to support service provisions that ensure opportunities of children to engage with their peers and to remain with their family, as opposed to be placed in group homes or other alternative living arrangements [1, 35]. A service provision policy that considers strategies to support inclusive, accessible social development can also in the long run increase the level of solidarity and social inclusion, ultimately contributing to alleviate poverty and marginalization for children with disabilities and their families [36]. The implementation of a social model of disability horizontally in the policy-making process could also promote the realization of human rights for other vulnerable groups such as immigrants, and direct the loci of responsibility of social inclusion and economic opportunities from government to the society as a whole [37]. Research suggests some actionable items in this area, including the implementation of universal design principles (i.e., equitable, flexible, simple and intuitive, perceptible information) at all levels of society. These principles address changes in the collective environment rather than at the individual level [38]. The concept of universal design in policy is that if policies are inclusive, specific policy for traditionally excluded groups would not be necessary.

A person-centered approach to disability means that an individual's unique needs are identified and that the individual is involved in plans to address those needs. The CRPD "confirms" the rights of children that were previously approved, not creating new rights but continuing what was gained, with special considerations for children with disabilities. The research literature and the CRC and CRPD support the creation of person-centered policies and service in a lifespan strategy [39, 40], considering that environmental obstacles become more significant as children age [41], and also considering the developing capacities of children, and evolving challenges they face over the life course to allow for thriving in all spheres of life [40, 42].

Actionable items supported by research in this area include encouraging services to consider the needs of children in the context of the family, and consider systems of care beyond the family to alleviate the excessive reliance on mothers for care [43]. Other solutions are to encourage caregivers to actively participate in decision- and policy-making processes [44, 45] and service evaluation [46, 47]. Families are the key stakeholders to the process and can suggest and affect changes in more efficacious manners than decision-makers who are not directly involved in caring for children. One area to note is the need for effective transition planning from childhood to adolescence and into adulthood, which is paramount to maximizing post-school outcomes in a rights-respecting perspective [48]. Transition supports should be considered at all spheres of care including health, education, and social services.

Lack of information is a frequently mentioned barrier extensively documented to accessing health care at different levels [44, 46, 49, 50]. At the provider level, research shows that staff in a variety of services is often poorly qualified to address disability issues. Lack of knowledge about best practices in disability supports is a barrier to adequate service provision [51].

Applying Rights-Based Approaches to Health Care and Research

A rights-based approach to health can play a critical role in equalizing all individuals. Regardless of the method being used to incorporate rights-based approaches in health promotion, one of the major challenges lies in the conceptualization of the motivation (why to incorporate rights into a health perspective), the expected outcomes or measurable end points (what to measure), and the process of implementation of rights-based approaches into the different health systems (how to measure) [52]. It is important to overcome these challenges in order to increase the practical application in research and practice.

Over the last few decades, rights-based approaches have mostly received rhetorical acknowledgment, but no implementation solutions in health promotion of individuals with HIV/AIDS [52, 53], mental health [54, 55], maternal health [56], and essential medicine (medicines that satisfy the priority needs of the population within the health care sector, specifically for disease prevalence, and evidence on efficacy, safety, and comparative cost-effectiveness) [57]. More practical aspects of implementation to rights-based approaches

have been proposed in other global issues not related to health—such as climate change [58], forest conservation [59], and fisheries governance [60]. Interestingly, use of RBA strategies in sectors other than health overlaps with the strategies identified in the review above: advocacy and awareness [59], capacity building [60], legal recourse [61], and creating the best environment [58]. Therefore, the basic principles and strategies to implement rights-based approaches to improve the well-being of individuals in different contexts of policy and action rest on a common ground. The implementation details will vary according to the context and the population, but common action mechanisms remain.

On contextualizing the use of rights-based approaches to improve the health and participation among children with disabilities, the existing evidence helps in pointing out how similar principles have been applied to different fields and domains. For example, rights-based approaches along with a social relations approach have been suggested to inform implementation of the "Least Restrictive Environment Mandate" in the education sector by fostering educational standards and mainstreaming specialized training for educators for teaching students with special education needs. The use of rights-based approaches also supports the manner in which different provinces or states provide inclusive education, health, and social services to children with disabilities and their families, as part of the mandatory, universally available public services [62].

Another example of rights-based approaches' use to inform practice behaviors of professionals engaged in promotion of health of children with disabilities is the development of a human rights based monitoring framework with indicators to support monitoring of rehabilitation services and programs [63, 64]. This framework was developed using a concept-mapping approach where an analysis of the obligations of States under the CRPD with regard to rehabilitation services served as a conceptual platform for identifying potential indicators of services in key areas. Stakeholders involved in this process included researchers, persons with disabilities, and rehabilitation and health care providers. They identified key areas that served to indicate the minimum commitment of the States in relation to their children, and as benchmark to rehabilitation service provision [63, 64]. The outcome was a valid and universally applicable set of indicators that are suggested to have profound impact on the design and implementation of evidence-based disability policies and programs, and to support countries in measuring performance through documentation of comparative information on rehabilitation care systems [63, 64].

In research, RBAs have been used as a basis to develop projects, conduct environmental scans, and to review programs and policies. Different examples exist to support the use of RBAs as a framework to promote the right to health in areas of health research. In global health research, one example was a case study conducted to evaluate the development of the programs and activities of a non-governmental organization (NGO), Plan International, a child-centered organization that works primarily in local communities in over 50 developing nations [65]. It showed that adoption of RBA strategies transformed the NGO's interactions with local communities, increased individual rights awareness, and resulted in greater ownership being exercised by community organizations [65]. On the other hand, the case study also revealed a limited ability of an NGO to address disparities and discrimination within local communities, as well as neglect of collaboration with domestic social movements and civil societies [65].

In rehabilitation science and education research, one study identified gaps in the integration of disability rights principles into allied health professionals' competencies and education [66]. The authors used RBA to develop a guide to evaluate the curriculum content and interview the course coordinators, and identified factors affecting the uptake of RBA in the education systems [66]. The study identified strategies such as increased collaboration between allied health professionals and curriculum renewal to promote the integration of disability rights in allied health education [66]. The study also identified that allied health professionals' curriculum was based largely on a biomedical approach rather than a social model of disability or an RBA [25]. The lack of use of this language as part of the rehabilitation professionals' capacity building is a gap that must be addressed. Rehabilitation practitioners are part of the professionals who are involved in the health promotion and health care of children with disabilities from early age through to the entire life span. Incorporating a solid notion of health as a right, and human rights frameworks as a foundation for health care providers' education can be transformative.

In national policy, a few examples exist of implementing rights-based frameworks into health policy, particularly in cases where litigation was used to demand for services or resources. In Canada, for instance, challenges to implement rights-based approaches exist at the federal level, considering the provincial jurisdiction of health services. The Canada Health Act narrowly defines what is included in publicly funded health care services (physician and some hospital-based services). However, not all services provided in hospitals have been determined to be medically necessary health services and therefore may not be covered by provincial/territorial health care insurance. In the case of autism, the federal government's stance is that it is the provinces and territories that determine which health services are necessary and which are components of behavioral interventions or social supports. Thus, caregivers have challenged provincial legislation appealing to provincial decisions around services such as Applied Behavior Analysis (ABA) and other early interventions for autism. One landmark case in British

Columbia (BC) was the Auton 2000 lawsuit [67]. Parents of children with autism argued that denying their children medically necessary ABA treatment was discrimination under the Canadian Rights and Freedoms Act. The case also built on the assumption that if ABA was provided through public health care, this would greatly improve access to ABA in the schools. This lawsuit was won in BC, and later struck down by the Supreme Court of Canada, making ABA services available in many different provinces [68].

Different areas of inquiry are calling their scholars and practitioners to act on what is called the broader research agendas, "grand challenges," or areas of societal impact that are beyond the traditional scope of the profession or research-specific focus. The use of human rights based frameworks is one of the most authoritative and internationally recognized principles recognized as a challenge through the United Nations Sustainable Development Goals as part of everyone's obligations to promote more just, equal societies.

Examples of areas that are not traditionally linked to human rights but have recently established human rights research agendas include international business [69], Internet governance [70], and economics [71]. These fields have brought together research evidence, scholars, advocates, and policymakers to address the intersections between these fields and human rights frameworks, and are contributing even to child rights in aspects spanning from child protection in online environments, to considerations of child development in growing economies, and building capacity for child and youth agency.

The examples highlighted above indicate the relevance and significance of using RBAs to inform the development of policies, programs, and supports, and call attention to the urgent need to bring this agenda to all fields of childhood disability research and practice. A recent special issue of the *Seminars in Pediatric Neurology* highlighted the role of Social Pediatrics in understanding social determinants of health in childhood neurology practice [18]. The WHO Commission on Social Determinants of Health has also highlighted the need to build capacity in research with a focus on health equity, including a better understanding of processes that affect people's experiences of health and health services, such as disability [72]. The gradual shift of health research from a focus on medicine and the life sciences and clinical studies, toward the understanding of the social determinants of health and more recently with an emphasis on systems and the people who use, affect, and are affected by them creates unique opportunities for health care professionals, trainees, and researchers to develop research that encompasses rights-based frameworks and contributes to these broader systems discussions. This type of inquiry has a tremendous potential to promote health in a new perspective, and make each encounter with the health system, with a health care provider, become an opportunity to exert the right to health.

The implementation efforts of rights-based approaches continue to be accompanied with elemental (education, training, awareness), organizational (administration, government), and financial challenges. It is understood that addressing these challenges will require a substantial amount of resources and coordination between professionals from multiple sectors; it is our hope that RBA is incorporated to develop programs and policies in real-world settings to improve the quality of life of children with disabilities and their families. For, as the WHO succinctly puts it, achieving health equity "is the right thing to do, and now is the right time to do it" [73, p. 3].

Conclusions

For children with disabilities to enjoy the highest attainable standard of health, there is an urgent need to direct resources and political will to the implementation of States' obligations under international law. As research has shown, and discussed in this chapter, a rights-based approach is particularly useful in this regard, and has shown to be successful in a variety of contexts. Such an approach centers around the needs of the individual child with disabilities, acknowledges the child's agency, and significantly holds out the responsibility of States throughout its layers of policy and agencies as well as society at large to implement the right. As societies are measured by their protection and support of those who are most vulnerable, working to appropriately include children with disabilities in the conversation is key.

Multiple Choice Questions

1. What would constitute a state's act of omission in relation to human rights?
   (a) **A failure to implement incremental steps toward the realization of Human Rights Conventions into its policies**
   (b) A lack of service offerings for a certain population
   (c) Misrepresentation of a specific minority group in the state's law
2. How is childhood disability represented in Human Rights frameworks?
   (a) In the theoretical framing of disability by the WHO
   (b) In policies that explicitly adopt a Human Rights approach
   (c) **In articles dedicated to children in different treaties such as the right to life health, education, and family**

3. How does the correlation between severity of disability and socioeconomic status relate to a human rights understanding of NDDs?
   (a) There is no relation severity of disability depends uniquely on biological factors
   (b) **The health outcomes of children with NDDs depend to a great extent on the characteristics of the environment where they are, which includes policies and societal values**
   (c) A low socioeconomic status implies individual risk factors leading to severe disabilities
4. Which are three key areas of health where children with disabilities are often neglected?
   (a) **Sexual and reproductive health, emergency responses to disasters, and health promotion initiatives**
   (b) Dentistry, neurology, and hematology
   (c) Early interventions, school-based interventions, and transition to adulthood
5. What are the guiding principles of the Convention on the Rights of the Children?
   (a) Equality and justice
   (b) **Non-discrimination; best interest of the child; the right to life, survival, and development; and the right to participate**
   (c) All children are entitled to live life with dignity, and all children should receive the same type of services and provisions for their development

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Patient Navigation: Core Concepts and Relevance to the Field of Pediatric Neurodisability

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Abbreviations

ASD	Autism Spectrum Disorder
BCAAN	British Columbia Autism Assessment Network
CDBC	Complex Developmental Behavioral Conditions
NDD/D	Neurodevelopmental Disorders and Disabilities

Learning Objectives

In this chapter, the reader will:

1. Understand the origins of and core concepts comprising patient navigation.
2. Appreciate the particular challenges experienced by children with neurodevelopmental disorders and disabilities and their families in accessing and coordinating supports and services.
3. Learn about innovative approaches to navigation for children with neurodevelopmental disorders and disabilities and their families.
4. Consider challenges and opportunities in the application of navigation to the context of children with neurodevelopmental disorders and disabilities and their families.

Highlights

- Patient navigation is primarily focused on: (i) barrier identification and reduction, (ii) facilitating individual and/or family connection to service, (iii) the coordination and integration of services across disparate sectors, and (iv) the provision of educational and emotional support.
- Children with neurodevelopmental disorders and disabilities and their families experience significant barriers in coordinating fragmented systems of support and services.
- Empirical research supports that patient navigation-based approaches are efficacious for facilitating access to assessment and support services for children with neurodevelopmental disorders and disabilities and their families.

Introduction

Children with neurodevelopmental disorders and disabilities (NDD/D) represent a significant cohort of the pediatric population, with a demonstrated need for coordinated external support. In order to effectively support their children and foster their development, caregivers must procure and manage specialized, yet fragmented, services that cross sectors and levels of health, social care and educational systems. The challenges associated with such a task should not be underestimated, as families' frustrations and concerns have been clearly documented in the literature. As such, there is a real opportunity for caregivers undertaking such activities to be supported by means of initiatives that aim to partner with families to coordinate disjointed services, find solutions to encountered barriers, and promote goodness-of-fit between child and family needs and available resources—in essence, someone or something to help the family "navigate" com-

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plex service systems. However, children with NDD/D and their families have been conspicuously left out of the bulk of the literature on patient navigation, a field that we suggest has great relevance to this population.

In what follows, we present an overview of the evolution of patient navigation as an area of practice and research, outlining the core attributes guiding such activities, as well as diversity in application. We then turn to the context of NDD/D, reviewing the limited work that has been done, and consider how we can apply lessons from other health contexts to this unique circumstance. We argue that consideration of patient navigation for children with NDD/D and their families is sorely needed, and share suggestions as to how we might begin to move this field forward.

Patient Navigation: Origins and Core Concepts

The importance of "patient navigation" was originally illuminated by the pioneering work of Dr. Harold Freeman, who found that an intervention pairing underserved breast cancer patients with free or low-cost examinations and patient navigation aimed at reducing barriers to care was associated with dramatic improvements in rates of early detection and 5-year survival [1]. Following this, work on patient navigation has proliferated across various health care populations (e.g., patients with HIV, diabetes, depression, addiction) and contexts (e.g., targeting patients experiencing socioeconomic-based health disparities or those in palliative care).

Given the range of settings within which navigation programs have been initiated, it is not surprising that an agreed-upon definition is lacking. There are, however, core principles that guide navigation delivery, though program elements are, and should be, context-specific. Central principles include being patient- (or family-)centered with a primary focus on successfully traversing and connecting fragmented service systems. It is also suggested that there be defined parameters around navigation delivery, including what the navigator's role, training, and scope of engagement (i.e., point of navigation initiation and resolution) should be [2, 3]. Ultimately, patient navigation seeks to provide education and emotional support, and remove barriers so that patients and families may experience meaningful and continuous engagement with needed health and community services, ultimately improving quality of life. The outcomes associated with navigation programs have been broadly conceptualized. For example, examined patient outcomes range from tracking changes in how individuals manage their health needs (e.g., medication and appointment adherence) to indicators of social wellbeing (e.g., financial stability, steady employment, and sufficient access to legal advice). Provider outcomes have included associated improvements in communication and coordination across health sectors, as well as increased knowledge, skills, and trust amongst program participants. Finally, health systems outcomes have been largely context-specific, such as reductions in emergency room or hospital visits [4–6].

The extent of the variability characterizing this field is evident in recent reviews, which highlight the vast diversity in how navigator scope has been operationalized. For example, in Carter et al.'s (2018) scoping review of 34 studies, patient navigators were identified by 15 different titles. Most navigator positions were filled by individuals, who may be lay persons (e.g., peer navigators), social workers, counsellors, or nurses, though some models employed interdisciplinary teams [4, 5, 7, 8]. In Carter et al.'s [4] review, lay persons served client populations whose needs were mostly material, such as those experiencing food and housing insecurity, or had challenges finding suitable employment. In contrast, multidisciplinary teams provided navigation for more complex patient populations. Patient navigators had a range of relevant experience, skills, knowledge, and education, though most received on-the-job training in which information about the target population was provided. Desired personal competencies included compassion, flexibility, and cultural awareness, among others (see [4]).

There is also diversity in the nature of navigator functions and activities. Valaitis et al. [6] found that the motivators for navigation program implementation could be categorized into three streams: improvement in the delivery of health and social services (e.g., ensuring improved programming and access to care); improvement in supporting particular health needs (e.g., primary health care needs among parents and children) or populations (e.g., patients with cancer or HIV); and improvement in quality of life and wellbeing. Within navigation programs, navigators addressed issues relating to health systems (e.g., obtaining health insurance and care providers, or identifying at-risk populations), aspects of disease (e.g., mental illness, comorbid conditions), social determinants of health (e.g., securing housing, food, and employment), or relating to patients themselves (e.g., providing education and outreach or helping to manage patient fears and beliefs) [4, 5]. Finally, navigators had a range of jurisdictions, with some services being limited to within-hospital coordination, whereas others facilitated hospital-to-community transitions or only assisted with connection across community agencies [7].

The fact that navigation programs have been implemented across such a wide range of health and community service settings indicates that the challenges associated with fragmented and disconnected services are not unique to any one disease or population context. We suggest that the lack of specificity around navigation definitions, principles, and

delivery models serves as both a benefit and a challenge. The advantage is found in the flexibility with which the core principles can be adapted to the needs of a particular patient population functioning within a particular health context. Conversely, this variability has challenged both research and practice, as literature describing and evaluating navigation programs has provided little detail about implementation activities or program fidelity, and has been undertaken in compartmentalized ways, such that initiatives build only on work conducted within the same health context. We therefore do not know about the extent to which such navigation programs have "reinvented the wheel," neglecting to apply "lessons learned" from one context to advance progress in another [5]. We now consider how the key navigation principles described can be applied to the unique context of children with neurodevelopmental conditions, a circumstance which, to date, has received little attention in the literature.

Patient Navigation and Children with NDD/D

Children with NDD/D represent a substantial proportion of the population, with prevalence estimates varying from 4.5% [9] to 1 in 6 children [10], depending on definition used and source of data. Like those who have most frequently been the recipients of navigation initiatives, children with NDD/D and their families also experience significant barriers in coordinating fragmented systems of supports and services. In fact, we suggest that children with NDD/D have service needs that stand out as unique, or at least distinctive, among individuals with health problems, which make them ideal candidates for these kinds of services. For example, children with NDD/D must negotiate amongst a complex array of services that cross care sectors (public and private), domains (health, education, social), and levels (primary, secondary, tertiary), often with limited funding obtained from distinct government ministries or other public sources, that each require ongoing management and reporting. It is also expected that individuals' service needs will continue across the lifespan, but in a dynamic fashion, as neurodevelopment interacts with ever-unfolding changes in environmental demands and expectations. This means that funding and services must be renegotiated at key transition points, such as upon school entry and reaching adulthood, when individuals "age out" of early intervention or pediatric services, respectively. The importance of the child and young person's family cannot be overestimated in relation to childhood neurodisability [11], so that services for this population need also to encompass the requirements of the family, and may include supports such as respite, counselling, and even material assistance for items such as specialized equipment.

Insights from Quality Improvement

The navigation needs of this population have been highlighted within a recent quality improvement initiative, in which families interacting with two specialized, multidisciplinary clinics at a tertiary care clinical and academic referral center were asked about their transition to community services after receiving a diagnostic assessment (see [12] for a more detailed description of the setting). In this western Canadian province (2017 population 4.8 million), the British Columbia Autism Assessment Network (BCAAN) provides standardized diagnostic assessment for possible autism spectrum disorder (ASD) in various sites; the Complex Developmental Behavioral Conditions (CDBC) Program similarly conducts diagnostic assessments of physician-referred children and youth with delays or abnormalities in multiple domains of development and/or learning, with or without specific risk factors such as genetic conditions or prenatal substance exposure, when specialized neurodevelopmental expertise is deemed more appropriate than secondary care developmental resources or specialty mental health services.

The mandate of BCAAN is primarily diagnostic; however, through this quality improvement initiative, an experienced clinic case manager conducted 103 interviews with families 6–11 months after their children had undergone an ASD assessment through an affiliated clinic. Families of children who received an ASD diagnosis (ASD positive; $n = 64$) were included along with those who did not (ASD negative; $n = 39$). The ratio of ASD positive-to-negative participants represented in the study is consistent with that of the larger assessment network from which participants were drawn. Interview questions were developed in collaboration with a few families who had been through the autism assessment pathway at the tertiary referral center, and inquired about family's perceptions of the overall assessment process, their ability to obtain community and school services post-assessment, and the need for follow-up. A consistent theme that arose during each component of the interview was families' desire for more information about and connection to community resources. When reflecting on needs during assessment, participants most frequently (33%) indicated a desire for more detail and concrete information about community resources and strategies for connecting with such services. This was expressed to an almost equal extent by those whose children did (36%) and did not (31%) receive an ASD diagnosis. Post-assessment, 84% of families indicated that they had outstanding questions, most frequently relating to a need for resources around how to build a team of service providers (59%), as well as around obtaining strategies to assist with their children's behavior and social-communication skills (57%). An overwhelming 93% of

participants indicated that there was a need for a follow-up process that would have the tertiary referral center connect with parents after the assessment was complete, with most (83%) recommending phone or face-to-face contact, and approximately half (49%) suggesting ideal timing would be 4 weeks post-assessment. Participants indicated that this follow-up contact should provide an opportunity to check-in with families about how they were doing, answer remaining questions, and share knowledge about how to access resources and build service teams [13].

In particular, the needs of ASD negative families were illuminated as unmet. For example, approximately one-third of those whose children were diagnosed with ASD indicated that the resources shared during assessment were helpful, whereas this was not noted by any of the participants whose children did not receive a diagnosis. Similarly, almost half (46%) of ASD negative families felt that they were not given sufficient information with which to obtain necessary community resources, as compared to only one-quarter of the ASD positive group, and as a result felt frustrated and alone post-assessment. Finally, only one-third of ASD negative caregivers indicated that they were able to obtain answers to questions they had following assessment, as compared to 59% of those whose children were ASD positive. As such, even though the developmental and behavioral challenges posed by ASD negative children were significant enough to meet criteria for a formal and specialized ASD assessment, they were uniquely isolated by insufficient connection to helpful community resources.

Within the clinic servicing CDBC Program patients at the same tertiary referral center, 15 families of children who received a diagnosis of fetal alcohol spectrum disorder (without comorbid intellectual disability) were interviewed in order to determine how the clinic team could improve upon the assessment process, and ultimately how well families were able to connect with community resources. Overall, families indicated that they were connected to community services, but at a shallow level. Many were still on waitlists for services, or supports were perceived as inadequate, particularly as related to availability within the school system. Transition to community post-diagnosis was therefore identified as challenging, with one-third of participants sharing their desire for greater clarification around service recommendations. Following this, recommendations for adapting clinic processes were made, including sending families a Frequently Asked Questions document prior to the family conference, during which diagnostic information and treatment recommendations would be shared, to help them know what to expect; asking families about what information they wanted to receive during the family conference; shifting the format of family conferences, such that the amount of information communicated would be less overwhelming; and receiving a follow-up call post family conference [14, 15].

Though specific to particular diagnostic assessment contexts, this work provides a snapshot of families' concerns, needs, and perceived barriers to accessing community resources and supports post-assessment, and highlights the role a navigator could play in ameliorating such challenges. Importantly, this work emphasizes the unique needs of children with complex neurodevelopmental conditions who may not receive a clear diagnosis but still have significant support needs, and likely have even greater challenges in linking with community resources. The themes that emerged provide evidence that specifying concrete steps and pathways for accessing needed services is of paramount importance throughout the diagnostic assessment process and beyond. Families consistently communicated their clear desire for follow-up, which would allow families to seek clarification regarding recommendations and to engage in problem-solving around encountered barriers.

Following this evaluation, the BCAAN implemented a program change aiming to better connect ASD positive families to available provincial resources. With consent, the contact information of ASD positive families is now shared with a provincial information center for ASD and related disorders whose mandate is to provide information on best practice treatments and supports, as well as training to families, service providers, and professionals. Under the change, this information center now contacts ASD positive families in the greater metropolitan region at 2 weeks post-assessment, with the intent of providing follow-up and to assist families in navigating provincial funding requirements, finding and hiring professionals, identifying key community supports, and managing developmental transitions. BCAAN also provides follow-up to families who did not receive an ASD diagnosis to try to connect them to appropriate services for their child in the community.

Insights from Empirical Evidence

Empirical research further supports that this is an area of substantial challenge for families of children with NDD/D, with caregivers describing the necessary support navigation and coordination demands as stressful and burdensome [16–20]. These barriers may be insurmountable for families who may be vulnerable in other ways, such as is the case for those of low socioeconomic status [21, 22] or who are immigrants [23, 24]. Despite the significant presence and coordination needs of this group, however, children with NDD/D and their families are often not the beneficiaries of navigation initiatives. For example, children with NDD/D have not been represented as the specific target population within any of the recently published scoping reviews, nor within an environmental scan examining navigation programs for children with complex health conditions [4–8, 25]. Rollins et al. [26],

however, have put forth a detailed conceptual impact model, which outlines the multifactorial ways in which patient navigation can address commonly encountered barriers facing this population, and the anticipated improved child and family outcomes (see Fig. 47.1).

The small amount of research reporting on programs for children with NDD/D confirms the potential in applying navigation principles in practice. For example, Guevara et al. [27] describe the Opening Doors to Early Intervention Program, which aimed to increase referrals to early intervention for young children with developmental delay. In this program, families met with a patient navigator to discuss their child and the nature of early intervention, and received a follow-up phone call or text message to identify and address any remaining barriers. The program resulted in improved rates of families completing a multidisciplinary evaluation, and in the proportion of children ultimately determined eligible for service. Similarly, Roth et al. [28] found that families who were paired with an Autism Navigator at the point of diagnosis, as compared to those who received navigation support three months later, were more likely to have scheduled or completed service appointments relating to medical, educational, therapeutic, and parent resource needs. Finally, Feinberg et al. [29] also report significant improvements in rates of ASD diagnostic assessment completion among low-income families from racial-ethnic minority groups randomly assigned to a Family Navigation intervention as compared to those receiving usual care (95% versus 58%, respectively). This confirms that patient navigation holds great potential for children with NDD/D and their families, though we do not know the extent to which such initiatives have been undertaken.

Another innovative initiative is the BRIGHT Coaching program intended for families of young children (18–54 months) on waitlists for developmental assessments and services across four Canadian provinces (British Columbia,

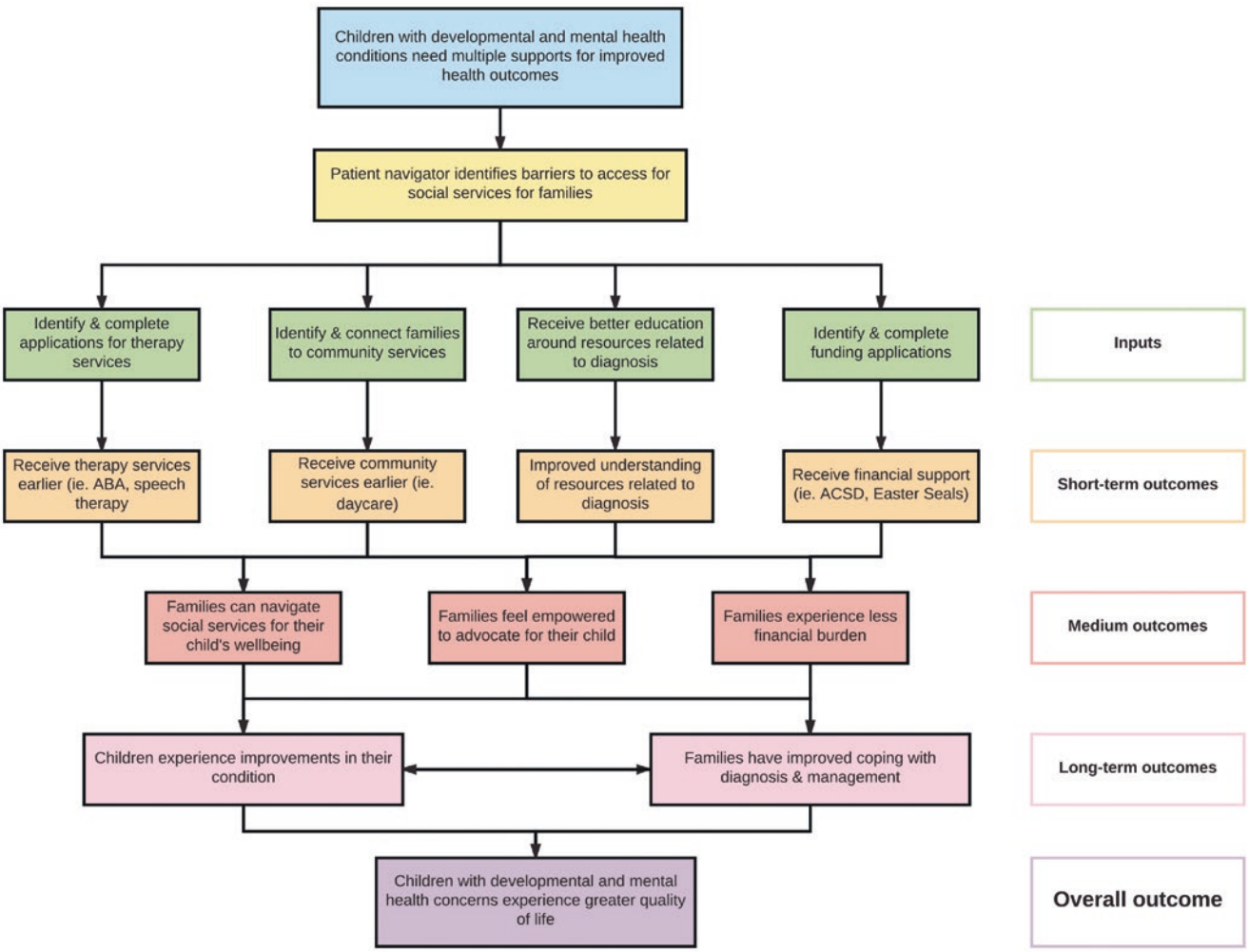

Fig. 47.1 Conceptual intervention impact model by Rollins et al. [26] (Reprinted with permission from "Rollins M, Milone F, Suleman S, Vojvoda D, Sgro M, Barozzino T. Patient navigators: Mapping the route toward accessibility in health care. Pediatr Child Health. 2019;24:19–22")

Manitoba, Quebec, and Nova Scotia). This program is unique in that it connects families who would not otherwise have access to NDD/D-specific services with supports, including: online education tools and resources about child development, service providers, family support, and community services; an online community discussion board where participants can network with other parents; and a coach who connects with parents by phone to guide the family in identifying areas of developmental concern, promote developmental stimulation, support mental, physical and family dynamics challenges, and to provide general information (see https://www.child-bright.ca/bright-coaching/ for additional information). The program was developed with stakeholder consultation about the "key ingredients" of coaching interventions, as well as in response to an online needs assessment survey completed by Canadian families in which they provided feedback about intervention design, logistics, and content. Within this survey, families indicated that access to coaching would improve caregiver mental health and emphasized their desires for training in advocacy and play-based skill development, as well as for personal support (i.e., related to stress management) [30]. The initiative's feasibility, acceptance, and impact are currently being evaluated using a multi-site pragmatic randomized clinical trial that compares the BRIGHT Coaching model to usual and locally available care for parents of children with emerging developmental delays [30].

NaviCare/SoinsNavi is a research-based navigation initiative based in New Brunswick, Canada, that was developed based on a province-wide needs assessment conducted with over 120 youth, families, and service providers. This program is innovative in that navigators, who consist of a registered nurse and lay person, serve both families of children and youth (aged 25 years and younger) with complex care needs (including those without a specific diagnosis), *and* care providers (see https://www.navicarenb.ca/ for additional information). Individuals connect with the service through a toll-free number, by email or Facebook messenger, and to date, the program has served over 100 families and care providers since its inception in January 2017. The navigators help to coordinate care, facilitate transitions, connect families with resources, and advocate for broader system change. Evaluation is ongoing, though initial findings indicate high rates of family satisfaction, and that families feel relief, reduced stress, and perceive improved care coordination and increased knowledge of available programs and services [31].

Navigation and its Alternatives

The above examples and evidence exist under the banner of "Navigation," but it is also pertinent to mention that other approaches are espoused, aimed at ensuring a more seamless experience of health and social services throughout the life journey of children with complex health and developmental needs. Within the United States health context, establishing and implementing the Medical Home concept has been advocated by the American Academy of Pediatrics and others [32, 33]. This model of care places community-based pediatric practices at the center; these Medical Homes are entrusted with ensuring that children and families receive preventive and follow-up care as per best practices and that referrals and connections to other services and supports are facilitated [32]. In the United Kingdom, there has been focus on interagency working and collaboration [34, 35] to bring together service providers in the health, social services, family support, and educational sectors. Innovative funding models, organized at a regional level, have been developed to achieve this goal [36]. At a more aspirational level, a family-centered, community-based system of services has been propounded to address the need for co-ordinated and integrated care and services [37].

Moving the Field Ahead: Challenges and Opportunities

As is true in many domains of health care, a one-size-fits-all approach is unlikely to succeed in the field of childhood neurodisability. The children and families are very diverse, and so are their needs from health, social and other service sectors. For example, some children's needs will center around medical, nursing and habilitative supports for mobility and feeding, and their safety in a setting like school. For others, supports for learning, socializing, and regulation of behavioral responses may be of primary concern. The role of the patient (or family) navigator would look different in these two circumstances. Despite this fairly obvious fact, however, it should be possible to set out the main aspects of the role in terms that are broad enough to cover most situations and which could form a set of "core skills and attributes/attitudes" of the navigator role within childhood neurodisability. Table 47.1 sets up a proposed list of this kind.

Another source of variability is that families differ in their perceived need for navigational (or care coordination) support, as has been found in an in-depth study of continuity of care for children with chronic health conditions. Some parents were open to the assistance of a person who could help to coordinate care, while others felt that this kind of activity was a parent's responsibility [38]. The lesson here is that the navigational supports (or support persons) should be made widely *available*, but organized in a way that plans for some families accessing them and others not.

Navigational supports may also look very different depending on whether families live in urban or rural areas.

Table 47.1 Core Roles of Navigator Within Childhood Neurodisability

Patient and family navigators
Provide:
Advocacy—For specific individuals and families, and for broader system change Emotional support
Informational/educational support
Facilitate:
Access to resources and supports (community, educational, medical, social, etc.)
Continuous and timely service engagement
Identification and reduction of barriers
Peer connections/networking
Transition planning

Whereas in an urban setting, a support person may be available for in-person meetings and service provision, in rural and remote areas, tele-health ("tele-navigation") may be more appropriate—something that warrants closer study, alongside other aspects of the increasingly important field of telehealth [39].

Another challenge for the field of patient navigation in the context of childhood neurodisability is the assumption that navigational support will help the child and family to connect to services that are appropriate for them, needed, and available once the family is "navigated to" them. For children with NDD/D, behavioral and emotional concerns are encountered frequently and cause considerable stress within the family unit [12]. Sometimes these behaviors are core parts of the neurodevelopmental condition (e.g., relentlessly repetitive behaviors among children diagnosed with ASD), and sometimes the behavioral concerns reflect less specific differences in brain functioning that underlie NDD/D conditions (including differences in cognitive areas such as flexibility or maintaining focus on a task); or non-specific manifestations of a child's frustration when they cannot readily communicate wants or feelings. Supports for distressing behavioral patterns, which broadly fall under the rubric of mental health interventions and supports, are often not easily accessible for children in the general population or for children with neurodisability [40, 41]. This situation is compounded by certain kinds of behavioral supports, such as Applied Behavioral Analysis, being available to children with a specific diagnosis, such as ASD, but not available otherwise even if such intervention would help [42]. Although precise data are lacking on the frequency with which parents turn to health care providers for help with behavioral problems and associated issues such as sleep difficulties, available information on the prevalence of such concerns among the childhood NDD/D population suggests that help-seeking, and therefore navigational-support seeking for such concerns, would arise frequently. The implication of this is that work on establishing navigational supports for children with NDD/D must proceed in parallel with efforts to ensure that resources are in place to address the behavioral and mental health needs of these children and families.

A final consideration is not unique to navigation in the field of childhood neurodisability, but follows nicely from the issue raised in the previous paragraph which can be summarized as: if services for children with NDD/D and their families were only organized into a more seamless system, would we need patient navigation at all? This question was raised with regard to the plight of adult patients with chronic health conditions trying to navigate the "complex, fragmented, arcane rather than being patient centered" world of American health care ([43], p. 75S), but should be asked equally in child health. If systems were (re)designed to be more seamless, connected and integrated, with the interests and needs of children and families placed at the center, as has been advocated [37, 44], it seems likely that patient navigators would not be needed. Although system redesign along these lines is not likely to be accomplished any time soon due to a host of entrenched and powerful factors, initiatives such as interagency working and the family-centered medical home, both described earlier in this chapter, as well as wrap-around approaches in complex and mental health care [45], can all serve as service delivery and organizational models worthy of emulation, wider adoption, and implementation.

Concluding Thoughts

In summarizing the diverse body of literation around "navigation," and reflecting on the distinct needs of those affected by NDD/D, it is clear that the core attributes of the concept can be readily applied to this context. In order to move the field forward in a substantive way, however, such that there is meaningful advancement in the science, but also importantly within this area of practice, we suggest that the literature be detailed in its description of the population, their particular needs and surrounding contextual influences, as well as inclusion of efforts at evaluation. A reality of this domain of work is that navigation initiatives are often undertaken under the banner of "quality improvement" within an individual institution or program, and not as a purely academic exercise. There is, however, an obligation for widespread knowledge dissemination to both researchers and research users around such initiatives, despite challenges associated with undertaking implementation and evaluation in a clinic setting versus the methodological rigor that might be expected with lab-based research. Surely, the sharing of navigation successes and challenges as applied in the real world allows others to consider contextual similarities and distinctiveness, and will serve to expand the broader field of practice.

Multiple Choice Questions

Q1. Patient navigation originated from an initiative serving low-income breast cancer patients in the 1990s, and has since been applied to the following settings and contexts:
(a) Patients with HIV/AIDS
(b) Patients in palliative care
(c) Patients with substance abuse
(d) All of the above
ANSWER: (d) All of the above.

Q2. Empirical evidence supports the efficacy of patient navigation for children with neurodevelopmental disorders and disabilities and their families, because:
(a) In most research studies utilizing patient navigation, the child's disability resolves, and services are no longer needed
(b) Relevant research studies report increased access to assessment and support services for the child with a neurodevelopmental disorder or disability
(c) Relevant research studies report improvements for caregivers, including in quality of life, knowledge, and service satisfaction
(d) Both b and c
ANSWER: (d) Both b and c.

Q3. Navigation is not the sole approach that seeks to connect children with neurodevelopmental disorders and disabilities and their families to services. All of the following are parallel models discussed within this chapter, with the exception of:
(a) Coaching
(b) Connecting
(c) Medical Home
ANSWER: (b) Connecting.

Q4. Patient Navigation, as applied to children with neurodevelopmental disorders and disabilities and their families, refers to a specific, manualized program that can be implemented regardless of healthcare or community setting.
(a) True
(b) False
ANSWER: (b) False

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Evaluating the Economic Impact of Neurodevelopmental Disability Intervention on the Family and Community

48

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Learning Objectives [1, 2]

- Understand the roles of economic evaluation in decision-making.
- Measure the costs and benefits of neurodevelopment disability intervention focusing on ASD, CP, and FASD.
- Describe the types of economic evaluation.
- Discuss the challenges of measuring and valuing the costs and benefits for the economic evaluation of interventions for NDD.
- Discuss important considerations and highlight areas for future work.

Highlights [1, 3]

- There is a need to identify or develop the most appropriate instruments for measuring and valuing the health outcomes of children with NDD.
- The economic evaluation should incorporate the loss of value of gross earnings resulting from NDDs for a child.
- A future economic evaluation of NDD interventions should measure and incorporate cost and health spillover effects on the family.

Introduction

Neurodevelopmental disability (NDD) is a heterogeneous group of conditions with onset in the first 5 years of life, characterized by impairments in personal, social, academic or occupational functioning [1–5]. Developmental deficits within this population vary from specific limitations of learning or control of executive functions to global impairments of social skills and intelligence. These deficits manifest in early development and are pervasive, affecting individuals throughout their lifespan [6, 7]. Among Canadian children with a disability, 75% of them also have an NDD [8]. Over 90% of children with NDD experience limitations in activities throughout their lifespan that impact their quality of life [8, 9]. The prevalence of NDD is estimated from 5% to 9% of all children in Canada [10–12]. The number of children diagnosed with NDD has increased in recent years; for instance, the estimated prevalence of autism spectrum disorder (ASD), one of the most common NDD, has increased dramatically in the past several years, from 1 in 150 in 2002 to 1 in 59 in 2014 in the US [13, 14]. The actual cause of this sharp increase is unknown, but the literature suggests it may be due to a combination of changes in reporting practices, diagnostic substitution, and increased awareness about NDD [15].

NDD has a substantial economic burden on families and welfare systems. Children with NDD have higher healthcare service utilization (three times more hospitalization, two times more physician visits than those without NDD) [12, 16] are more likely to fall in the top 5% of most frequent health care users (43% of children with developmental delay are high users), are more prone to mental health problems (85% of persons with ASD have a mental health disorder over their life) [17–19], and are more likely to make use of government disability support programs than children without NDD [20]. Furthermore, the needs of children with NDD are heterogeneous, even within a single diagnosis, with varying support needs over their lifespan. For instance, treatments across the lifespan of an individual with ASD might

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need a multi-disciplinary approach starting from the early intensive behavioral intervention (EIBI) or home-based educator in early childhood, special education during school years, and employment support or accommodation in adulthood. With these complex needs, coordination of disability services and supports is critical to ensure that children with NDD receive access to the right services at the right time to support their healthy growth and developmental trajectories and to support their opportunity to participate in society in meaningful ways.

Despite 82 countries ratifying the United Nations Convention on Rights of Persons with Disabilities (UN CRPD), the primary responsibility for coordination of services lies with individual families [8]. As a result, caregivers of children with an NDD face higher financial costs [21–23], lower quality of life [9, 24–26], and higher rates of poverty compared to other caregivers who do not have a child with NDD [8]. Families caring for a child with a disability have reduced working hours and labor force participation, with an estimated average annual out-of-pocket cost of $10,000 to $30,000 to the family, without including the additional costs covered by insurance [16]. Respite for children with complex needs requiring 24-h care was estimated at $131,000 per year, including reduced working hours and labor force participation [27].

From a public funding perspective, individuals with NDD are supported through multiple ministerial areas: community and social services, health, and education. A challenge is often fragmentation of services offered to support children with disabilities and different priorities, mandates, and approaches to service delivery [28]. Barriers to the coordination of care include a lack of consistent policies and inconsistent standards for information sharing across the continuum of care and distinct eligibility criteria [29, 30]. In line with these findings, families with children with NDD report greater challenges to accessing support services compared to other families [31, 32]. Families report a feeling of being "bounced around" from one service to another, and this results in psychological toll; they contend with significantly higher levels of stress, feelings of isolation and frustration, and physical and mental health issues [33–37].

The remarkable progress has been made in terms of diagnosis, understanding risk factors, treatments, and providing support to an individual with NDD in the last decade. With high burdens associated with NDD, every intervention that is efficient, effective and accessible cannot be implemented. When resources are limited, decision-makers are increasingly turning to evidence generated by economic evaluation to decide on the resource allocation, but little evidence exists regarding economic evaluations of NDD interventions [26].

Economic evaluation in healthcare compares the costs and consequences (the term "consequences" is also referred to as "outcomes," "benefits," or "effects") to two or more interventions, of which primary objective is to provide information to healthcare decision-makers on efficient use of limited resources to maximize health benefits [38]. It can inform healthcare decision-making processes at many levels, from national and provincial decision-making bodies to decision-making bodies at the local level.

The purpose of this chapter is to provide background information and highlight some of the opportunities and contextual challenges associated with the economic evaluation of NDD research and interventions for this heterogeneous child population. First, we highlight the importance of determining the perspective and time horizon of the evaluation, emphasizing the consideration of family and caregiving effects. We then provide an overview of what relevant costs and outcomes need to be measured. We describe existing economic evaluation methodologies and their applicability to NDD's interventions, focusing on ASD, cerebral palsy (CP), and fetal alcohol syndrome (FASD) as illustrative NDD. Finally, we discuss emerging opportunities and challenges for the economic evaluation of NDD as a call to action for researchers and clinicians.

Considering the Perspective and Time Horizon for an Evaluation

Evaluation Perspective

Before gathering data on costs and outcomes associated with research and interventions, it is necessary to specify the perspective and time horizon of the study. The perspective of economic evaluation for NDD interventions provides a framework for analysis and determines what costs and benefits to include in the analysis. Possible viewpoints for an evaluation could include society, ministry of health or other government perspectives, service providers (hospital, nursing home, and community service providers), families, patients, or agencies [38]. The societal perspective is considered as the gold standard in economic evaluations as it captures all the costs including healthcare costs, parental out-of-pocket costs, productivity losses of individuals with NDD and their parents, and social service costs and consequences incurred during the provision of intervention. However, evaluations designed to answer resource allocation questions often take a government perspective. For example, the cost-effectiveness of expanding an Intensive Behavioral Intervention (IBI) program for children with ASD in Ontario was performed from a government perspective concluding that expansion of IBI to all eligible children in Ontario can save total costs of $45,133,011 (in 2003 Canadian dollars), and gains in dependency-free life years [39].

Often much of costs for NDD occurs in sectors outside the health care system in terms of social assistance, educa-

tion (special education needs), and family impact (disrupted parental employment and household expenses) [27, 40]. A systematic review of the economic consequences of child and adolescent mental illness including ASD and developmental delays in the UK found that the largest costs are from outside the health care system[1] such as social service, education system, criminal justice system, and productivity costs [41]. For example, a cost-benefit analysis of early intensive behavioral intervention (EIBI) for children with autism or pervasive development disorder-not otherwise specified (PDD-NOS) found cost savings ranging from $187,000 to $203,000 per child for ages 3–22 and from $656,000 to $1082, 000 per child for ages 3–55 years [42]. Notably, the majority of costs saving were from schools (special education, intensive education costs), home and community-based services, family support services, and income from regular or supported work. The National Institute for Health and Care Excellence (NICE)and the second US panel on cost-effectiveness in health and medicine encourage researchers to think broadly about the inclusion of costs and outcomes in the evaluation [43, 44].

The notion that children (patients) are not "isolated individuals," but rather have a social circle of parents, siblings, other relatives, and friends, has emerged in recent years [45]. A child's health can have positive and negative impacts on the quality of life and economic well-being of the family. There are two main effects characterized for family members described as the "caregiving effect" and "family effect" [46, 47]. Caregiving effect comprises formal (paid) caregivers or informal caregivers. An informal caregiver is often a family member (i.e., a mother, father, or both) for a child with NDD. The family effect refers more broadly to the impact of the child's health on the quality of life and economic well-being of family members, applying to parents, siblings, and other relatives living in the house. The family effect is purely related to the social relationship with a child and does not include caregiving effects. Examples include the influence of NDD of a child on the health and well-being of his/parents or siblings or grandparents. The family and caregiver effects are commonly called as "spillover effects" in economics and can be categorized into costs spillover effects and health spillover effects.

Raising and caring for a child with NDD can have a significant financial burden on families called as *costs spillover effects* [48–52]. The time required to care for children with NDD combined the high cost of specialized childcare may reduce parent's ability to sustain paid employment, resulting in substantial productivity losses for family. Parents may need to seek supports and pay out of pocket for their mental health problems caused high caregiving demand or higher levels of psychological difficulties caused by the disability of a child.

Moreover, meeting high care demand and diverse needs of services for children with NDD requires much time, effort, and patience. This often results in psychological distress, depression, anxiety, and mental health problems in family members called as *health spillover effects* [53–58]. Evidence from literature has shown that parents to children with NDD are at a higher risk of anxiety, depression, and sleep disorders than family members of typically developing children [59–61] do. Results from a qualitative study suggested that a higher level of stress is due threat to parents' freedom, professional career, personal relationships, and parents' worries about their child's future and treatment [62]. Unaffected siblings of children with NDD are also vulnerable to experiencing withdrawal, aggression, depression, and anxiety due to inability to lay with their siblings, aggressive and annoying acts of their siblings, and caregiving responsibilities [63]. A meta-analysis on siblings of children with a chronic illness and found that siblings of children with a chronic illness scored lower in peer activities and developmental cognitive scores compared to the controls [64]. The detail on the measurement of spillover effects on parents can be found in Sect. 7.

Effective intervention can have an important effect on the quality of life and socioeconomic well-being of both the child (with NDD) and their caregivers and family members. It is also evident that a child's resource use is influenced by the parent's care-seeking decisions. Furthermore, a parent's well-being is also likely to be affected by the illness of their child, especially when the illness is severe, even if they do not provide informal care [65]. When conducting economic evaluation from a societal perspective, these costs and effects should be taken into consideration [66]. Adopting a societal perspective assesses whether introducing the (NDD) intervention will yield greater welfare to society and is an efficient use of resources.

Time Horizon and Discounting

The time horizon is the length of time that the costs and the benefits are accrued in the economic evaluation. It should capture the length of the program and the associated costs and benefits of the intervention. Many individuals with NDD need continuing supports for their entire lives. Guidelines for economic evaluation encourage the use of a lifespan approach when possible [43, 67]. For example, early intervention for children with ASD (as shown by Motiwala et al. 2006 [39]) may lead to gains in dependency-free life years over a lifetime. Other outcomes for children may include greater workforce participation in adulthood, higher income, and less dependence on social assistance [68]. Thus, the longer time

[1]Only 6% of the costs fall on the health system

horizon—a lifetime horizon for children with NDD—would be preferable as it includes the impacts on a child's future productivity and quality of life. However, a short-term time horizon may be more relevant in some cases, depending on the objectives of the intervention and how realistic these types of projections are.

The costs and benefits occurring in the future need to be discounted for future value. Discounting is performed in economic evaluation to reflect time preference [69]. Discounting is very important in NDD economic evaluation because often we have to create a long-term and lifetime models to capture the effects of interventions. For consistency, discount future costs and effects at the same rate.[2]

Measurement of NDD Costs

Costing involves the systematic collection and estimation of the use of the resources within the health sector by NDD children and their families, resource utilization in the sector outside the health care and productivity changes. The process of cost estimation can be categorized into three steps: *a) the identification of relevant resources that are (will be) consumed, b) measurement of resources consumed*, and *c) valuation of the resources* [38]. Broadly the measurement of costs can be divided into either bottom-up or top-down approach. The bottom-up approach involves identifying and collecting all the resources that are used to provide a service to NDD children, assigning a monetary value to each resource consumed, then calculating the total unit costs. The top-down approach involves first calculating the total cost of service consumed at the system level, and then breaking down into the unit costs [70]. When estimating costs of NDD interventions, data on service utilization and other economic effects often are drawn from outside the health care setting [16, 26]. This is because children with NDD (will) use a range of public services outside the clinical setting such as transportation, housing, vocational training, and the criminal justice system, to name a few.

Costs (broken into *direct costs* and *indirect costs)* comprise the total net expenditures on intervention and are input into the burden of illness studies and all methods of economic evaluations. Table 48.1 provides examples of the costs associated with different perspectives.

Table 48.1 Examples of costs associated with perspectives: Broad perspective and Narrow perspective

Costs	Societal	Government (provincial or federal)	Service providers (hospital, nursing home, community service providers, etc.)
Inpatient days and outpatient visits	Yes	Yes	Yes
Professional fees	Yes	Yes	No
Prescription drugs	Yes	Yes	No
Community service/ programs	Yes	Yes	No
Education (special education, behavioral, speech and language, occupational therapies)[a]	Yes	Yes	No
Out-of-pocket expenditures	Yes	No	No
Productivity losses/ days off work (individuals with NDD, parents and caregivers, loss of usual activities)	Yes	No	No
Social assistance (disability)	Yes	No	No

[a]Note that many therapies for NDD children are also offered by private health care organizations

Direct Costs

Direct costs include medical costs of health care resources consumed (such as costs of the physician, nurses, medications, speech and language therapies, assistive devices and residential care) [38] and costs of non-medical goods, services, and other resources consumed (such as the costs of special education, transportation costs for taking children to the hospital or other health care centers) in the provision of interventions or in dealing with side effects or other current and future consequences linked to an NDD[38]. Direct costs of the NDD intervention can nominally be taken from direct expenditures on the intervention, but they must be augmented with projections of ultimate costs from implementation to the end of the intervention. For a prospective study, information about resource consumption/utilization of health care services by NDD could be derived directly from data collection (routine visits, telephone interview, mail out questionnaires, patient diaries, and administrative office of service providers). For a retrospective study, information can be obtained from secondary sources such as administrative databases, hospitals/physician chart reviews, or peer-reviews articles. The unit cost of resources used can be obtained directly from participating organizations and surveys or indirectly from the schedule of benefits, federal or provincial government reports, and peer-reviews articles. The total cost of resources consumed would be the quantity of the resources used multiplied by the unit cost. Out-of-pocket costs for parents or other family or caregivers can be measured by asking them to estimate average weekly expenses because of NDD child's health.

[2]Discount rates vary from country to country; mostly between 3% and 5% [44].

Indirect Costs

Not all costs are expenditures. NDD have high indirect costs for children with NDD, parents, family members, and other caregivers, and indirect costs are typically captured as productivity costs. Productivity costs are the loss of value of gross earnings of an affected individual or their parents (caregivers) or the family members resulting from a child's disability. Productivity loss is also called "absenteeism" and "presenteeism." Absenteeism refers to the loss of productivity due to absence from work because of health-related issues, while "presenteeism" refers to reduced productivity at work due to health-related issues. Often productivity costs related to paid work are calculated based on basic data on a patient's absence from work.

Caregivers (parents/family members) of NDD children have to quit their job or reduce working hours or change the jobs to provide support and care. For instance, it has been estimated that 85% of individuals with ASD need some measure of care and assistance from their parents and families for the duration of their lives [71]. Furthermore, the employment stability of family members in the household, including parents and siblings, is also affected by the health of a child with NDD. Evidence indicates that employed parents of children with chronic disability including NDD are more likely to go in late to work or leave early from work, reduce working hours, or quite a job to provide care to an affected child compared to parents of typically developing children [26, 52, 72, 73]. In addition, families of children with NDD must incur out-of-pocket expenses associated with specialized equipment, transportation, and medications.

The loss of productivity could also arise from the premature death of children with NDD calculated as the present value of future earnings that can be expected over the person's lifetime (at least until retirement) if they had not died prematurely [74]. Moreover, children with NDD have enormous difficulties in getting a job and keeping as they transition from school to employment. They often experience adversities in finding a job because of lack of social skills, adequate training, lack of appropriate working environment and education, or they are not productive as someone without NDD at the workplace [75–77]. Consequently, there is a significant productivity loss due to NDD.

There are mainly two approaches to calculating the indirect cost: the human capital and the frictional cost approach [78]. The human capital approach estimates the productivity cost of work absence as the value of forgone earning as a result of illness, disease, disability, or death [79]. It uses the number of hours missed from work by the person and then multiplies that by the hourly wages of that person. For example, the productivity cost of a caregiver of an NDD child is the total number of hours missed to provide support and care multiplied by the caregiver's hourly wages. For people who are not employed, hourly rates can be calculated using individual characteristics such as education, age, and gender. The friction cost approach estimates the productivity cost as the cost (time) needed to restore production to its original level [79]. For instance, if the parents quit their job to care for a child with NDD, the productivity cost is the time and resources needed to restore the production to the level before she/he quit the job [27]. In addition, parents may also need to give up their leisure time, personal care time, or regular household activities time to provide care to children with NDD. There are no standard methods on how to calculate the costs of loss of usual activities. The human capital approach can (i.e., hourly wage relevant for labor) can be used to estimate leisure trade-off. However, researchers must be cautious about overestimation.

Measurement of NDD Effectiveness

Effectiveness refers to the outcomes of the intervention. The outcomes should primarily reflect the experience and well-being of children with NDD who are treated, supported, or offered service. For example, a study evaluating the cost-effectiveness of CBT compared to treatment as usual (TAU) used the percentage of children free from their primary anxiety disorder and quality of life years (QALYs) as outcomes measured [80]. Using a broader perspective, this study could have included outcomes for families (parents), caregivers such as reducing the stress of caregivers and increasing the productivity of parents of children with NDD.

The measurement of health outcomes in children with NDD involves assessment of physical, social, psychological and cognitive functioning abilities. Ideally, these measures should be based on direct, NDD children's experience. The health outcome in children with NDD can be measured using disease or condition-specific instruments or general health outcome profile instruments or generic preference-based HRQoL. Because of feasibility, reliability, and validity issues in children, proxies such as parents, caregivers, and health care professionals are often used. The health outcomes in economic evaluations for NDD are frequently measured as the quality of life (QoL)[3] and health-related quality of life (HRQoL).[4]

[3]The Quality of Life (QoL) is a multidimensional instrument that measures individual's subjective perceptions of well-being across all the domains of life: physical, cognitive, psychological and social functioning. The WHO definition of QoL is "the individual perceptions of their position in life, in the context of culture and value systems in which they live, and in relations to their goals, expectations, standards and concerns."

[4]Health-related quality of life (HRQoL) only encompasses aspects of quality of life that directly influence the health of individuals or populations. Unlike QoL, it does not include broader concepts such as income, jobs, housing, education, spiritual, environment, and political and personal freedoms. There are different methods of measuring HRQoL and can be broadly divided into specific measures, general health profiles measures, and preference-based measures.

Economic Evaluation of Interventions for Children with NDD

Cost of Illness (COI)

When there is a need to evaluate the economic burden of disease, cost of illness (COI)[5] is used. COI is frequently used in a public health policy setting to argue which diseases should be given a higher priority in the policy agenda setting. COI studies can be classified into the incidence and prevalence-based studies based on the data used. Incidence-based estimates use the lifetime costs of a condition first diagnosed with NDD in a particular year, whereas prevalence based-studies estimate the total costs of NDD over a particular period, regardless of the time of diagnosis [81]. With the high associated burden estimates, these are common in NDD [82–85] compared to other methods of economic evaluation (CEA, CBA, CUA, or CMA). For example, very few studies have examined the economic evaluation of interventions of FASD; the focus has primarily been on burden estimates of the disorder [83]. COI is very helpful to demonstrate which NDDs may need more attention and allocation of prevention or treatment resources.

While there are studies estimating economic cost based on specific clinical conditions or disorders, estimates of economic costs associated with childhood disability delineated in functional outcomes (according to the International Classification of Functioning, Disability and Health (ICF)-based social model of disability approach) range depending on the severity of the disability and the estimation strategy used. There is a direct relationship between the level of severity of NDDs and economic costs [24]. The main drawback of COI estimates can be difficult to compare depending on what is included as burden estimates will vary greatly depending on whether only health care costs or other indirect costs such as productivity loss is included [86].

Types of Economic Evaluations

There are mainly four different methods of economic evaluation, including cost-minimization analysis (CMA), cost-benefit analysis (CBA), cost-effectiveness analysis (CEA), and cost-utility analysis (CUA). The identification and estimation of various types of costs are similar across all these approaches; the feature that distinguishes these types of economic evaluations is the way in which outcomes are assessed [38]. Here we will briefly overview when each methodology is used with corresponding outcomes.

[5]The cost of illness is also called as a burden of disease (BOD).

Cost Minimization (CMA)

When the outcomes of two treatments or programs are equivalent, and the intervention is reducing the cost of the identical outcomes, cost minimization (CMA) is used. CMA for NDD is typically used in the context of human resource planning or drugs with equivalent effectiveness. For example, CMA was used to compare equivalent types of Botulinum Toxin Type A (Botox® and Dysport®) in the treatment of spasticity in pediatric cerebral palsy (CP), finding Dysport® can save money on total annual costs per patient [87]. A more recent study estimated the cost of clinical exome and genome sequencing using a bottom-up micro-costing approach [88]. The study compared the costs associated with three different diagnostic tests (chromosol microarray analysis, whole exome sequencing, and whole genome sequencing (WGS)) from a payer perspective over 5 years for children with ASD, finding that WGS was more expensive. This approach is not commonly used in NDD research as interventions do not typically have equivalent effectiveness in outcomes.

Cost-Effectiveness Analysis (CEA)

When comparing different alternative interventions that have the same outcome of interest, cost-effectiveness analysis (CEA) is used. In a CEA, the consequences of treatments or programs are measured in natural units such as life years gained, functional improvements, dependency-free life years or any other disease-specific outcome. CEA compares the cost per unit of the outcome, typically expressed in a ratio (called an Incremental Cost-Effectiveness Ratio (ICER)[6]) reflecting the extra costs required to achieve a unit of particular effectiveness, such as the cost per improvement in IQ or cost per dependency-free life years gained. The main advantage of using CEA is that it focuses narrowly on the clinical outcomes that are familiar to the clinicians and patients[38]. A major weakness of CEA is its inability to compare different interventions with multiple or different outcomes [38]. For example, a study using CEA of an ASD intervention used dependency-free life years (DFLYs) as an outcome [89]. The study compared two pre-diagnosis interventions-Denver Model (ESDM-I) and pre-diagnosis parent-delivered ESDM (ESDM-PD) with the Ontario Status Quo (SQ). From the societal perspective, the EDSM-I created an additional 0.53 DFLYs for $45,000 less than SQ. The evaluation indicated that pre-diagnosis ASD-targeted intervention might save money from a societal perspective. The DFLY out-

[6]The ICER is the ratio of the change in costs of the intervention compared to the alternative such as doing nothing or using the best available alternative intervention to the change in the outcomes of the intervention. An intervention that costs less and has a better outcome than the next best alternative is considered as the best choice or dominant.

come measured in this study does not allow for comparison with other interventions which have different outcomes other than DFLYs.

Both general health profile measures and disease-specific measures are used in CEA. For a summary of examples of general health profile measures and preference-based health-related quality-of-life measures applicable to children with NDD, see Tables 48.1 and 48.2 in Lamsal et al. [29]. The main disadvantage of general health profile and disease-specific measures is the lack of standardization into a scale that can be decoded into QALYs or estimates of the amount of time in health weights by a preference score of utility [90]. The QALY is a metric that combines the QoL and life years gained into a single outcome. The QALYs are calculated by multiplying years of life by health utility value. Health utility can be defined as the preference of patients or the general population of given states of health defined by the descriptive system, interpreted on a scale, "1" represents full health and "0" indicates a health state equivalent to dead. Negative values are possible representing health states worse than dead. Some general health profile or disease-specific measures can be converted into QALYs using mapping or the use of an algorithm(s) to predict health state utility values using data on other indicators or measure of health [91]. It allows predicting health utility scores using data on general health profile measures or disease-specific measures to estimate QALYs. Algorithms for mapping general health profiles and disease-specific measures into health utilities have been reported for some conditions. However, very few algorithms have been conducted specifically for children with ASD, CP, and FASD.

General Health Profile Measures

General health profile measures provide a comprehensive dimension of health-related quality of life directly related to the disease's symptoms and characteristics including physical, psychological and social health dimensions. Numerous instruments can be used to study NDDs. Table 48.2 focuses on the measures that are most relevant to NDDs with the primary focus on three conditions: ASD, FASD, and CP. There is variability in the instruments, and important considerations highlighted in Table 48.2 include the number of items in the instrument, applicable age range, child or parent reporting, validity/reliability in ASD, FASD, CP, and mapping to generic preference-based measures.

ASD, CP, and FASD-Specific Measures

Disease-specific measures focus on health outcomes related to a specific NDD. CP QoL Questionnaire for children [92], (CPCHILD) Child Health Index of life with disabilities [93], Pediatric Quality of Life Inventory-CP module [94], and DISABKIDS-CP module [95] are some examples of disease-specific measures for CP. Autism Diagnostic Interview-Revised (ref) and Autism Diagnostic Observation Schedule [96] are some of the ASD-specific measures. More information on health outcome measures for children with ASD can be found in Payakachat et al. 2012 [97]. We are not aware of any specific or diagnostic measures for FASD.

Cost-Utility Analysis (CUA)

Where there are multiple outcomes to consider or outcomes of alternative interventions differ, finding a common denominator across different outcomes is one approach that is often used. The health outcomes in CUA are expressed in a single matrix such as quality-adjusted life years (QALYs), disability-adjusted life years (DALYs), and health year equivalents (HYE). The outcomes are expressed as a measure that reflects how an individual's value or gain utility from an intervention. Using an outcome measure such as QALYs is appealing as it provides a universal metric that allows comparisons of health outcomes among different patients, populations, and times or comparison of interventions for NDD with other physical and mental health intervention in both child and adult populations[38, 90]. Like CEA, CUA can be expressed in an incremental ratio called a "cost-utility ratio" and compared with a threshold ICER. Commonly used ICER thresholds are $50,000 per QALY gained in the US [98], £20,000–£30,000 per QALY gained in the UK [99], and $20,000–$100,000 per QALY gained in Canada [100]. However, in some cases such as treatments of rare or chronic disease, interventions with very high ICERs might be considered. CUA is the recommended type of economic evaluation in guidelines across the world, including Canada, the UK, and Australia.

Preference-Based HRQoL Measures

There are various methods of measuring utilities for health states, and these methods can be broadly grouped into the direct preference-based and the indirect preference-based approaches. The direct approach is based on mapping preferences directly into a utility scale. Standard gamble (SG) [101], time trade-off (TTO) [38], and rating scale (visual analog scale, [VAS]) [102] are the three most commonly used techniques in direct measurement studies. For example, one study compared the costs and outcomes of continuous intrathecal baclofen infusion (CITB) to the standard treatment in children with intractable spastic cerebral palsy [103]. Health effects were measured from a health care perspective with a time horizon of 1 year using a visual analog scale ultimately converted into QALYs, concluding that CITB is cost-effective based on the Netherlands's threshold willingness to pay per QALY.

However, children with NDD often lack cognitive and linguistic skills, social and communication skills, and adap-

Table 48.2 Examples of general health profile-related quality of life measures for ASD, CP, and FASD. Reproduced with permission from: Lamsal et al. Economic Evaluation of Interventions for Children with Neurodevelopmental Disorders: Opportunities and Challenges. Applied Health Economics and Health Policy, 2017 Dec;15(6):763–772

Instruments	Domains	Number of items	Age	Raters	Has reliability/ validity has been tested for ASD, CP, and FASD?	Has been used in children with ASD, CP, and FASD?	Mapping available for children with ASD, CP, and FASD?
Child Health and Illness Profile (CHIP): CHIP-CE & CHIP-AE [117]	CHIP-CE: Satisfaction, comfort, resilience, risk avoidance CHIP-AE: Satisfaction, discomfort, risk avoidance, and resilience	45 and 108	CHIP-CE: 6–11 years CHIP-AE: 11–17 years	Child self-report/ proxy report (parent)	Not found	Yes—ASD [118]	Not found
Child health questionnaire (CHQ): CHQ-PF28, CHQ-PF50 &CHQ-CF87 [119]	Physical functioning, role/social limitations, general health perceptions, bodily pain/discomfort, family activities, parent impact, mental health, self-esteem, general behavior, family cohesion and change in health	28, 50, and 87	CHQ-PF28: 4–11 years CHQ-PF 50:5–18 years CHQ-CF87: 10 years or older	Child self-repot/proxy report (parent)	Yes—CP [120]	Not found	Not found
Functional status II-R [121]	Communications, mobility, mood, energy, play, sleep, eating, and toileting patterns	43 and 14	0–16 years	Child self-repot/proxy report (parent)	Not found	Not found	Not found
Generic Children's quality of life measure (GCQ) [122]	Worry, happiness, relationships with parents, general satisfaction, support, health/appearance, attainments	25	6–14 years	Child self-report	Not found	Not found	Not found
Health Status Questionnaire [123]	Malformation, neuromotor function, seizure, hearing, communication, vision, cognitive and other physical disability	8 clinical domains	2 years or older	Proxy report (parents, health care professionals)	Not found	Not found	Not found
KIDSCREEN: KIDSCREEN-52, KIDSCREEN-27 & KIDSCREEN-10 [124]	Physical Well-being, psychological well- being, moods and emotions, autonomy, parent relations and home life, peers & social support, school Environment, bullying, and financial support	52, 27, and 10	8–18 years	Child self-report/ proxy report	Yes—CP & ASD [125]	Yes—ASD [126]	Yes—general adolescents (KIDSCREEN-10 to CHU 9D) [127]
KINDL Questionnaire [128]	Psychological Well-being, social relationships, physical functioning, everyday life activities	24 and disease-specific module	3–17 years	Child self-reported/ proxy report (parent)	Not found	Yes—CP [129]	Not found
Pediatric quality of life inventory (PedsQL™) [130]	Physical, social, emotional, and school	23 and 35	2–18 years	Child self-report/ proxy report (parent)	Yes—CP & ASD [131, 132]	Yes—ASD [133]	Yes—ASD [134]
The inventory of measuring quality of life in children and adolescents (ILK questionnaire) [135]	School, family, social contact with peers, interests and recreational activities, physical health, psychological health, overall assessment of the quality of life, exposure to diagnostic and therapeutic	9 thematic areas	6–18 years	Child self-report/ proxy report (adults and their medical doctors or therapists)	Not found	Yes—ASD [136]	Not found
The TNO-AZL questionnaires for children's health-related quality of life (TACQOL) [137]	General physical functioning/complaints; functioning: Motor, daily, cognitive, social, global emotional (negative and positive)	56 and 43	6–15 years	Child self-report/ proxy-report (others administered by parents)	Not found	Not found	Not found

Table 48.3 Examples of preference-based health-related quality of life (HRQoL) measures for ASD, CP, and FASD. Reproduced with permission from: Lamsal et al. Economic Evaluation of Interventions for Children with Neurodevelopmental Disorders: Opportunities and Challenges. Applied Health Economics and Health Policy, 2017 Dec;15(6):763–772

Instruments	Domains	Number of items	Age	Raters	Has reliability/ validity has been tested for ASD, CP, and FASD?	Has been used in children with ASD, CP and FASD?
16 dimensional (16D) [138]	Mobility, vision, hearing, breathing, sleeping, eating, speech, excretion, school and hobbies, mental function, discomfort and symptoms, depression, distress, vitality, appearance, and friends	16	12–15 years	Child self-report	Not found	Not found
17 dimensional(17D) [139]	Mobility, vision, hearing, breathing, sleeping, eating, speech, excretion, school and hobbies, discomfort and symptoms, depression, vitality, appearance, friends, concentration, anxiety, learning, and memory	17	8–11 years	Child self-report	Not found	Not found
Child health utility 9D (CHU9D) [140]	Worried, sad, pain, tired, annoyed, schoolwork/homework, sleep, daily routine, and ability to join in activities	9 dimensions with 5 levels of response options per dimension	7–11 years	Child self-report	Not found	Not found
Euro QoL five-dimension questionnaire for youth (EQ-5D-Y) [141]	Mobility, looking after myself, doing usual activities, having pain or discomfort, feeling worried, sad or unhappy	5 dimensions with 3 levels of response options per dimension and 100-pints VAS	4–11 years (4–7: Proxy version, 8–11: Self-report)	Child self-report/ proxy report	Not found	Not found
Health utilities index (HUI): HUI mark 1, HUI mark 2 & HUI mark 3 [142]	HUI2: Sensation, mobility, emotion, cognition, self-care, pain, and fertility HUI3: Vision, hearing, speech, ambulation, dexterity, cognition, and pain	15 and 40	5 years and older (5–8 years, 8–12 years and 13+ years)	Child self-report/ proxy report (parent)	Yes (ASD) [143]	Yes—ASD & FASD [144]

tive and behavioral skills. These deficits can affect a child's understanding of health and well-being. Using direct approaches such as SG or TTO can be problematic. Also, children at a young age do not have the capacity to understand the concept of time in a way that would allow them to trade risks against time spent in various health states. Given these difficulties, the indirect approach (also called multi-attribute measures) measures have been developed to bypass the measurement task by using one of the pre-scored multi-attribute health status classification systems that exist. For example, a study in children with CP found that intrathecal baclofen delivered via an implantable pump was cost-effective within the traditional ICER threshold of $50,000 per QALY [104]. This study constructed QALYs from a panel of expert clinicians (rather than children with CP) who used the Health Utilities Index-2 to rate health states connected to courses of treatment. CUA is difficult in child health and NDD evaluations due to challenges associated with measuring utility (discussed later), as well as obtaining an accurate estimate of life years gained for various interventions in this population. In particular, there is a need for a valid and reliable measure of utility for calculating QALYs in children [26]. Table 48.3 describes examples of indirect preference-based instruments for CUA relevant to ASD, FASD, and CP.

Cost-Benefit Analysis (CBA)

Another approach to bringing different outcomes together is to express them all in monetary units as a common denominator. This approach, called cost-benefit analysis (CBA), is used to identify whether the monetary value of the benefits exceeds its costs. CBA can be used to compare outcomes across conditions and also useful in its ability to capture the "spillover effects" or the health and non-health benefits to individual and others (family, friends, and relatives) of NDD interventions. CBA can be used to compare the interventions across the sector such as health, social, environment, and others. A key challenge with CBA lies in valuing health benefits from NDD interventions such as human life and quality of life in monetary terms.

Measurement of Family Effects and Caregiver Effects

A few methodological approaches for measuring spillover health effects of caregivers and family members have been introduced in the literature [45, 105, 106], but we are not aware of studies utilizing these methods to capture the spillover effects in NDD. Studies in other disciplines have used these approaches to capture spillover effects. Using the standard gamble approach, Prosser et al. asked family members of Alzheimer/dementia, arthritis, cancer, and depression patients to value the spillover effects [107]. They found that the effects of illness were extended to other members of the family. Another approach to direct elicitation is to ask caregivers to how much time they would trade-off at the end of their remaining life expectancy to prevent a childhood condition. Ultimately, QALYs can be estimated by dividing the amount of time the parent is willing to trade by the remaining life expectancy.

In contrast to direct measures, many studies have used indirect methods to measure spillover effects. For instance, Brouwer et al. and Poleij et al. have used EuroQol-5D to measure the health-related QoL of RA caregivers and parents providing informal care to young patients with congenital anomalies [108, 109]. The benefits of using preference-based measures are that it can be easily converted into QALYs and can be directly incorporated into economic evaluations. An alternative approach for measuring the spillover effects on the caregiver is using the Care-related Quality of Life (CarerQol) [110]. The CarerQol instrument was developed to measure the care-related quality of life in informal caregivers. More information about CarerQol can be found in Brouwer et al. 2006 [110].

Challenges for the Economic Evaluation of NDD

The challenges in conducting economic evaluations of the pediatric population have been acknowledged. Lack of a valid and reliable child-specific measures [111], complex and changing attainment during development [65], uncertainty of proxy measures (by parent or caregivers) [112], difficulties in measurement of spillover effects [45], difficulties in measurement of costs [65], and the need to capture the lifetime costs and consequences [38] are majors impediments researchers are facing in economic evaluation of child health interventions. Below we will discuss some of these complexities in the context of economic evaluations of children with NDD.

Important Considerations when Choosing Outcome Measures

The measurement of health outcomes in children with NDD involves assessment of physical, social, psychological and cognitive functioning abilities and health-related quality of life (HRQoL). Objective measures of physical function, laboratory tests, and biological markers can be problematic for children as there is a range of attainment during development. Children undergo dramatic changes in growth and functions that a failure to achieve normal functioning might not be indicative of developmental deficits observed later in life. An NDD child's health is interwoven with social determinants of health and defining health outcomes is often influenced by factors such as socioeconomic status, ethnicity, physical environment, biological determinants, and behavioral responses [113]. Complex and changing dependency relationships with parents, relatives, friends, teachers, neighbors, and community have made more difficulties in measurement and classification. Another challenge is that NDDs frequently have co-morbidities such as epilepsy and mental health concerns [114].

Moreover, each disorder has a unique etiology with wide heterogeneity of symptoms; domains related to a child's social, emotional, independence, communication and self-control functioning might not be present in instruments [107, 115]. Finding a single generic or preference-based instrument to measure the health outcomes of all NDDs is unlikely. For instance, ASD is characterized by repeated deficits in social interaction, communication, and behavioral functioning, whereas CP is a condition that involves primarily impairments in body movement and control. Many of the general health profile and preference-based measure instruments used in economic evaluations are designed for other general diseases or acquired from adults. Therefore, these instruments do not contain all domains that are applicable to the NDDs. For example, the Child Health Questionnaire (CHQ), KIDSCREEN and Child Health, Illness Profile (CHIP) and Quality Well-Being (QWB-SA) have a significant number of items related to social dimensions such as school, family, and peers; however, measures like the PedsQL and EQ-5D-Y have more items focus on physical and emotional functioning.

Using direct elicitation methods such as standard gamble (SG), time trade-off (TTO), and rating scale could be problematic as children with NDD often lack cognitive skills to understand the concepts of health and well-being. Often researchers have no choice but to use a proxy as a reporter. While proxy reporters such as parents or caregivers can be effective for observable signs and symptoms, they are less

accurate for subjective measures such as quality of life, emotion, and utility [112]. In addition, parent's views are affected by their health status, as well as their knowledge, experiences, and expectations [116]. In particular, there does not exist a valid and reliable preference-based measure of utility for calculating quality-adjusted life years (QALYs) of children including NDD, as noted by the lack of utilization of these instruments highlighted in a recent scoping review [90]. Researchers and clinicians should be aware of the theoretical and methodological purpose of their study before making decisions about which instrument to use for economic evaluation.

Important Considerations when Incorporating Caregivers and Family Effects

While numerous methods to incorporate the caregiver and the family effects have been suggested in the literature, there is a lack of standardized methodology. Researchers should be aware of challenges in measuring the spillover effects of caregivers and family members due to NDD child's health. For instance, separating normal caregiving and caregiving due to child's NDD is a challenge. Similarly, it is very difficult to know whether the particular effect (explicitly or implicitly) on caregivers or family members is due to the health of a child with NDD. There is a need for studies incorporating family and caregiver effects into economic evaluations for NDD interventions.

Important Considerations when Estimating Costs

Apart from identifying costs, measuring costs is also a challenge for NDD interventions. Children with NDD use a wide variety of services outside the traditional care delivery settings. This means that compiling and quantifying services in monetary terms can be challenging. Another difficulty includes measuring productivity costs for parents, caregivers, and NDD children. First, for parents and caregivers, it is hard to differentiate the hours of care provided from the usual care (if parents had a child without NDD). Second, it is difficult to think about productivity in children what future earnings the child would have if he or she had not NDD. Early interventions could have larger benefits in adulthood in terms of employment and reduce services use (hence reduced the long-term costs). Failure to incorporate the benefits that occur for the adult due to early intervention in economic evaluation potentially underestimates the effectiveness of interventions.

Conclusions

NDD children present unique measurement challenges for economic evaluation tools, as they often lack cognitive, communication and social abilities to respond to questionnaires on health and well-being. Often proxy measures are used; however, their reliability and validity are unknown or less representative. There is also a complex and evolving dependency relation with parents and caregivers that impacts the health outcome assessment of NDDs in children. Currently, no gold standard measures exist to evaluate the health outcomes of children with NDD.

There is an urgent need for identifying or developing the most appropriate instruments or methods that take into consideration these measurement challenges in this population. Preference-based or generic outcomes measures that have been used in the NDD research to the date are either derived from general diseases or adults and do not encompass all domains related to NDD. Efforts need to be invested in testing their reliability and validity in this population. Using disease-specific measures might be an option. However, this limits the ability to compare with other interventions. The disease-specific measures are preferable to general health profile measures if mapping to preference-based measures is available.

Inclusion of family and caregiver effects, as well as consideration of future productivity gains and losses for children with NDD due to the intervention, is necessary for conducting the economic evaluation. Several approaches and methods have been proposed in child and maternal health arena, which are applicable to NDD evaluation. A collective approach, "*family perspective*" that recognizes complex interdependency relationships between a child with NDD and quality of life and economic well-being of family members, may be appropriate for the NDD intervention. The development of valid, reliable and feasible methods for a family-centered approach to the measurement of related health outcomes and health care resources use is essential. Further research on methods to predicate and estimate NDD child's productivity is needed. Understanding the uniqueness of NDD child health, etiology of NDDs, patterns of health care need, and resources use, and dependency relationships with family members and caregivers and their impact on the measurement of costs and consequences will enable more representative economic evaluations.

Multiple Choice Questions [4, 5]

1. Economic evaluation in healthcare compares ———— of two or more healthcare interventions.
   (a) Costs
   (b) Benefits
   (c) Health outcomes
   (d) **Costs and Benefits**

2. Which of the following statement is true?
   (a) A societal perspective is broader and includes all costs and effects regardless of where, when, or on whom they fall on, including productivity losses of children with NDD and their family members and effects on the health and well-being of family members
   (b) A public health payer perspective includes healthcare costs borne by the publicly subsidized health program related to NDD, such as a provincial government program for children with NDD
   (c) A family perspective includes costs borne by the family of children with NDD
   (d) **All of the above statements are true**
3. The cost-benefit analysis differs from the cost-effectiveness analysis in that
   (a) **Costs and benefits are expressed in monetary units**
   (b) The health outcomes are measured in the quality-adjusted life years (QALYs)
   (c) The cost-benefit analysis is used when the consequences of two treatments or programs are equivalent and compare the costs of two treatments or programs
   (d) Both a and c
4. In the cost-effectiveness, the health benefits are measured in
   (a) Life years gained
   (b) dependency free-life years
   (c) Functional improvements
   (d) **All of the above** **Funding** We gratefully acknowledge the contributions of Kids Brain Health Network funded through the Networks of Centers of Excellence program.

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Part VII

Resources

Using Large-Scale Population-Based Data

49

Rubab G. Arim and Dafna E. Kohen

Learning Objectives

1. Describe, compare, and contrast primary and secondary data.
2. Categorize quantitative and qualitative data.
3. Summarize advantages and disadvantages of secondary data.
4. Define large-scale population-based databases.
5. Justify the value of large-scale population-based databases.
6. Differentiate cross-sectional and longitudinal surveys.
7. Distinguish between survey and administrative data.
8. Recognize advantages and disadvantages of survey and administrative data.
9. Describe and justify the value of record linkage.
10. List some examples of available secondary data in Canada to study children and youth with neurodevelopmental disorders.
11. Locate, select, and access relevant secondary data for a research question related to neurodevelopmental pediatrics.
12. Prepare and apply criteria to assess the appropriateness of a secondary data source for data analysis in neurodevelopmental pediatrics.

Highlights

- Large-scale population-based secondary data are essential and offer a major advantage to study issues related to neurodevelopmental pediatrics. Yet, they also have important limitations to consider for research use.
- In Canada, we have various large-scale population-based surveys and administrative data sources to inform research targeting children and youth with neurodevelopmental disorders. Researchers should apply criteria to assess the appropriateness of a secondary data source to their research question.
- The secondary data sources that are presented in this chapter are not exhaustive rather the information intends to showcase the potential use of available secondary data sources to study child and youth neurodevelopmental disorders. Similarly, the presented criteria are not intended to describe "best methods" but to depict options.
- A comprehensive picture of information required for broad research applications in neurodevelopmental pediatrics can be achieved by combining different databases using a process known as record linkage.

Introduction

Data are important to understand neurodevelopmental disorders in children and youth, to improve quality in their health care, to support policy and program initiatives, and to report on progress toward national and international reporting targets in this field. There are various ways of collecting data. Data collection strategies can involve *primary* or *secondary data*.

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Primary data are data that are collected for a specific research purpose [1]. For example, researchers might be interested in studying self-esteem in children with neurodevelopmental disorders. To this end, researchers can collect primary data in the form of *quantitative* (e.g., structured survey) or *qualitative* (e.g., open interview) data designed to examine self-esteem in a sample of children with neurodevelopmental disorders. The collected data would be entered into a database for later analysis to interpret the data and understand the findings. A quantitative database includes variables that are coded with a range of possible numeric values. For example, self-esteem scores can be coded based on a completed parent, teacher, or self-reported questionnaire on a scale ranging from 0 to 4 with higher scores indicating higher self-esteem (see [2] for an example) whereas a qualitative database can include documents, interviews, and audio or videotapes. For example, transcripts of an audiotaped interview could focus on how children describe and perceive themselves (see [3] for an example). When primary qualitative or quantitative data are made available for use to study research questions other than the purpose originally intended for data collection, it is then called secondary data [1].

Thus, *secondary data* are data originally collected for a different purpose and then used to address new and different research questions [1]. For example, data originally collected to study self-esteem in children with neurodevelopmental disorders may be used to measure sex and age differences in perceptions of physical appearance among children with neurodevelopmental disorders. Indeed, secondary data may come from various sources, including previously collected research data but also could be derived from other sources, such as administration, surveillance, management, claims, or planning data. For example, physician claims data originally collected to provide payments to physicians for their services can be used to identify children with neurodevelopmental disorders based on diagnostic information such as the International Classification of Diseases (ICD) coded by physicians [4] and to establish the cost for diagnoses related to neurodevelopmental disorders [5].

The main advantage of secondary data over primary data is that the data already exist so the researcher does not need to collect them. Often, other advantages include a large sample size, representativeness of the data, and reduced likelihood of bias due to recall or non-response [6], particularly when using large-scale population-based data. However, there are several important disadvantages of secondary data including restricted content, quality issues, and the necessity to tailor research questions to fit the available previously collected data.

The restricted content issues arise from the fact that researchers have little to no control over the subject matter area when secondary data are used and often secondary data may include breadth but not depth of specific topics [7], especially if they were not specifically designed or collected for the topic of interest such as neurodevelopmental pediatrics. For example, secondary data such as physician claims could include billing information (i.e., a code for services provided by practitioners along with information about paid amount for service). These data would also include information on diagnostic codes that may allow the identification of children with neurodevelopmental disorders but may not provide information about functional impairment or severity of the disorder and certainly would not include specific measures of self-esteem. Thus, in some cases, key variables on a specific research topic may be missing in secondary data sources and consequently research questions may need to be tailored to available data. In this case, the focus of research would need to rely on previously collected content, for example, mental health, if that was included, rather than self-esteem in children with neurodevelopmental disorders. Other cases may necessitate changes in deriving variables of interest given available data. For example, in the absence of information on functional impairment, daily activity limitations and participation restrictions, or severity in physician claims data, neurodevelopmental disorders can be identified with recorded information on the diagnosed condition such as epilepsy, cerebral palsy, and fetal alcohol syndrome based on included diagnostic codes. Conceptualizing neurodevelopmental disorders based on select neurodevelopmental conditions (e.g., cerebral palsy and epilepsy) that have similar consequences such as the need for a wide array of professional services and significant health care expenditures may be a plausible strategy given available information in physician claims data (see [8, 9] for examples). Overall, secondary data may be limited in covering a specific research topic in depth or covering various relevant aspects and thus may not be the optimal choice for certain research questions in a specific topic. A good practice to deal with restricted content issues of secondary data is to understand the background of the data (e.g., why collected, where they come from, how they are collected, and whether they are comparable to other data) and to assess whether they are "good enough" to accurately capture the concept and the population of interest.

While restricted content issues may result from the previous content development (for the primary data), quality issues may be related to the method of data collection and data entry, which are also not under the control of researchers using secondary data [6]. For example, data from some subgroups of populations of interest may be excluded (e.g., children with rare neurodevelopmental disorders or very young children), some variables of interest may not be included, an "imperfect" measure may have been chosen (e.g., a non-standardized measure when a standardized version exists), or coding errors may exist. In addition to understanding the purpose, population of interest, sample size, and variables in the data, a good practice to deal with quality issues of secondary data is to have an analytic plan that includes quality checks in its design. For example,

understanding the flow of the questionnaires, acknowledging the skip patterns, and keeping track of the total and subgroup sample sizes would be important practices to ensure an understanding of the sample and the data quality. Equally important is to have transparency and rigor in analyzing, interpreting, and reporting the findings from secondary analyses. Despite these issues, secondary data can save time and money for data collection and offer unprecedented opportunities for research that can contribute to understanding the lives of children and youth particularly those with chronic health conditions such as neurodevelopmental disorders.

In this chapter, we focus on two different types of secondary data sources: large-scale population-based survey data and administrative data. *Large-scale* means having a large scope of impact and is not defined by size. Large-scale data include a rich source of information with broad research applications. Note that the data can be primary or secondary and the sample size does not need to be "large" [7]. However, in the case of secondary data, large-scale data often include national or jurisdictional data with a large sample size, which can be used to answer a broad range of research questions. *Population-based* data can be described as representative of a well-defined population of interest [7]. At the national level, population-based data are gathered based on data collected consistently across a country usually based on representativeness of certain characteristics of interest. Representativeness means that the characteristics of the sample proportionally reflect specified characteristics in a target population. These characteristics are often part of the sampling strategy or the design of the survey sample. For example, a sampling strategy that ensures that all ethnic, gender, and economic groups are represented in proportion to national statistics in the database [7] would ensure that the size of the samples would be large enough to represent the characteristics of the population of interest. In addition, oversampling may be required to ensure appropriate numbers of respondents.

In the following sections, we use illustrative examples from large-scale population-based survey and administrative data in Canada and highlight the strengths and limitations for studying neurodevelopmental disorders in children and youth. We also provide examples from the work our research group has conducted on studies that have used population-based data sources to inform research specifically targeting children and youth with neurodevelopmental disorders.

Large-Scale Population-Based Survey Data

Large-scale population-based survey data are typically national or jurisdictional surveys with a large sample size and with results that are generalizable to the population of interest. *Cross-sectional* surveys are conducted at one point in time and thus provide snapshots of the population whereas *longitudinal* surveys are conducted at multiple points in time and thus allow for a "video" of the population such that events can be ordered and associations can be examined over time [10]. All surveys are designed to collect specific information at a specific point in time and are often administered online or by trained personnel via phone or in-person. In Canada, we have various large-scale population-based surveys that are collected, housed, and made available by Statistics Canada, the national statistical office to inform research targeting children and youth with neurodevelopmental disorders (see Table 49.1 for examples of Statistics Canada surveys).

Table 49.1 Examples of available large-scale population-based survey data collected, housed, and made available by Statistics Canada to study children and youth with neurodevelopmental disorders

Survey data	Survey design	Survey information
Canadian Community Health Survey (CCHS)	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&SDDS=3226
Participation and Activity Limitations Survey (PALS)[a]	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&Id=30014
Canadian Survey on Disability (CSD)	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&SDDS=3251
National Longitudinal Survey of Children and Youth (NLSCY)[b]	Longitudinal	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&SDDS=4450
Canadian Health Survey of Children and Youth (CHSCY)	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey.SDDS=5233
Ontario Child Health Study (OCHS)	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&Id=185995
General Social Survey (GSS)[c]	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurveySDDS=4502
Longitudinal and International Study of Adults (LISA)	Longitudinal	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&SDDS=5144
Indigenous Peoples Survey (IPS)	Cross-sectional	http://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey.SDDS=3250

[a]The PALS has been replaced by the CSD in 2012
[b]The NLSCY has been inactive since 2009
[c]The GSS includes a series of independent surveys, each covering one specific topic. The information above represents the GSS-Caregiving and care receiving

A prime example is the Canadian Community Health Survey (CCHS), which is designed to gather information about health status, health care utilization, and health determinants for the Canadian population 12 years of age and older living in the 10 provinces and the 3 territories ([11] for sample exclusions). The CCHS has four components. The core content and the theme content are collected from all respondents; however, while the core content remains stable over time, the theme content alternates from year to year. The optional content is uniquely prepared for each province and territory in coordination with health regions and varies from year to year to address the changing policy needs at the health region level. There is also the rapid response content that is collected for organizations requiring quick turnaround time and that are interested in an emerging or specific population health issue. The CCHS has also been supplemented by other focused cross-sectional content such as Nutrition or Mental Health in order to allow for reporting and responding to fulfill stakeholders' specific information needs. Notably, although the CCHS collects information on restriction of activities and functional status (optional content in CCHS), which may be used as a proxy for disabilities, a comprehensive identification of neurodevelopmental disorders for children and youth may not be possible because specific diagnosed neurodevelopmental conditions, such as epilepsy, cerebral palsy, and learning disability, are not asked in the content of the CCHS. Thus, the CCHS, as a general health survey, is limited in its ability to identify children and youth with neurodevelopmental disorders due to the limited age range of the sample (12+) but also the exclusion of chronic conditions that can be identified as neurodevelopmental disorders. Yet, the CCHS may be useful to examine information about the functional status of children and youth, which may be of interest to researchers since it includes items on the Health Utility Index (HUI; [12, 13]) or Washington Group—question sets (see Washington Group Short Set on Functioning at https://www.washingtongroup-disability.com/question-sets/wg-short-set-on-functioning-wg-ss/) depending on the year of data collection.

Table 49.1 presents some of the other available large-scale population-based survey data, which are collected, housed, and made available by Statistics Canada to study children and youth with neurodevelopmental disorders (see [14] for a more comprehensive review). To access data, Statistics Canada offers several options. For example, through the Data Liberation Initiative (DLI), a partnership between participating postsecondary institutions and Statistics Canada, faculty and students in Canadian educational institutions have unlimited access to public use files of numerous population-based survey data.[1] In addition, the Canadian Research Data Centres (RDCs) allow researchers to access a wide range of population-based surveys in a secure university setting. Similar to the RDCs, the Federal Research Data Centre (FRDC) provides a secure site where federal government employees can have access to a wide range of population-based surveys. Please visit https://www.statcan.gc.ca/eng/help/microdata for information about data access options offered by Statistics Canada.

Large-scale population-based surveys may serve as a powerful tool for researchers to study issues related to topics in neurodevelopmental pediatrics. However, population-based surveys are costly and require substantial time for content development and collection, and place a burden on respondents. Time is also required by the analysts to understand, process, and code the data prior to undertaking data analysis. Yet, large-scale population-based surveys such as the CCHS can serve multiple purposes including estimating the prevalence of general disability based on functional status in youth (but not young children). Large-scale population-based survey data also offer powerful data to examine social determinants of health such as gender, education, income, housing, and disability and the complex interactions among these factors for children with neurodevelopmental disorders and their families. Social determinants of health refer to a specific group of social, economic, and environmental factors, including but not limited to income, education, childhood experiences, and social supports and coping skills within the broader range of determinants of health [15, 16]. Social determinants of health strongly influence health and development [17], in particular, of children growing up in poverty and having adverse childhood experiences [18, 19]. For example, self-esteem of children with neurodevelopmental disorders may be influenced by various individual (e.g., gender) and family sociodemographic characteristics (e.g., income and education). Overall, large-scale population-based survey data may help to shed light on health disparities among children and inform policies and practices that foster the lives of children with neurodevelopmental disorders and their families.

Large-Scale Population-Based Administrative Data

By definition, administrative data refer to information gathered for administrative and not research purposes [20]. Governments at all levels, hospitals, and other organizations often collect data for record keeping, registration, transactions, and service delivery [21–23]. In Canada, both federal and provincial government departments collect administrative data such as tax, justice, health, education, and social services records. In the past, these data have been primarily used to produce statistics to inform decision-making for pol-

[1]The DLI allows access to Public Use Microdata Files (PUMFs), which contain anonymized individual level data.

icy, management, and practice. Currently, however, there is an increasing interest to access these data for research; in particular, there is an extensive use of administrative data in the area of health research. Administrative health data can include a wide range of records such as enrollment or eligibility information for programs and services, physician claims information, and managed encounters, including hospital services, professional services, prescription drug services, and laboratory services [22, 23]. All provinces and territories in Canada have administrative health data that include hospitalizations and physician claims [24].

In 1994, the Canadian Institute for Health Information (CIHI) was established to create a common approach to Canadian health information (see https://www.cihi.ca/en). Today, CIHI provides essential information on Canada's health systems and the health of Canadians. Yet, administrative health databases are mostly jurisdictionally managed (see Table 49.2 for some examples of administrative data sources in Canada). Indeed, there are many provincial resources that facilitate research using linked administrative health data. For example, in British Columbia (BC), Population Data BC (PopData) is a multi-university resource that offers one of the world's largest collections of health care services and population health data on BC's residents, as well as opportunities for education and training services on how to best use these data to study the determinants of human health, well-being, and development (see https://www.popdata.bc.ca/). PopData currently manages 21 databases from 2 federal and 6 provincial sources, including but not limited to Medical Services Plan Payment Information (MSP), Hospital Separations, Vital Statistics, and the Income Band data. There are also national registries such as the Canadian Chronic Disease Surveillance System (CCDSS), which is a collaborative network of provincial and territorial surveillance systems supported by the Public Health Agency of Canada (PHAC). The CCDSS includes health insurance registry data linked to physician claims and hospitalization data (as well as prescription drug data if available) from each province and territory (see https://health-infobase.canada.ca/ccdss/Index). Recently, there has been an attempt to combine multi-jurisdiction health data ([25]; the Pan-Canadian Real-[26]) through the PRHDN that includes researchers and data organizations from across Canada with an aim to create a unified infrastructure to advance pan-Canadian population-based research.

Administrative data can be appealing to researchers because they are already gathered and include a large amount of information. Nevertheless, researchers who choose to use administrative data for their research should also consider the limitations of administrative data. For example, identification of neurodevelopmental disorders in Canadian administrative health data is often based on diagnostic codes in hospital separations or physician claims; yet, these codes are often not evaluated for their accuracy or quality before being used in a research study [6, 27]. It is important to keep in mind that in the administrative data these codes are collected for billing rather than research purposes and therefore their validity is unknown. Indeed, previous research has shown that codes that are well-reimbursed (i.e., codes for a higher cost of service) are more likely to be coded compared to those that are not [20, 28]. In addition, codes that represent secondary reasons for clinical visits (e.g., comorbidities) are not always included (see [27] for an investigation of Down syndrome). Another well-recognized limitation of administrative data, particularly for neurodevelopmental pediatrics research, is the lack of information on functional status to identify neurodevelopmental disorders [29]. Information on social determinants of health may also be restricted in administrative data. For example, hospital separations often do not include information about socioeconomic status such as income or education. Finally, administrative data may be limited to broad outcomes such as health care utilization. Despite these limitations, large-scale population-based administrative data have increasingly become a resource for researchers to study issues related to topics in neurodevelopmental pediatrics.

Table 49.2 presents some of the administrative data available in various centers across Canada, including information on data access that may be on a cost-recovery basis. Generally, researchers submit a data access request form and the center responds on a cost-recovery basis. For example, data from CIHI can be retrieved at an aggregate or individual level on a cost-recovery basis whereas available linked administrative data at Statistics Canada (e.g., Canadian Vital Statistics Death Database linked to the Discharge Abstract Database [hospital separations], National Ambulatory Care Reporting System) can be accessed free of charge by university-based researchers (i.e., internal users) through the RDCs (but for external users access is approved on a fee-for-service basis). Note that procedures for data access may also vary. For example, while researchers can access administrative databases in BC on a secure research environment, in Manitoba, researchers are assigned to an analyst from the Manitoba Centre for Health Policy, who conducts the analyses in-house under secured conditions, and in Ontario, administrative databases can be accessed internally by scientists affiliated with the Institute for Clinical Evaluative Sciences. We advise contacting each center to request updated information about their services due to potential technological changes, changes in data access procedures, or unexpected events such as the Coronavirus Disease-2019 (COVID-19) pandemic.

Administrative data are large-scale in nature and typically cover a large period of time and population-based complex quantitative data, which are not always feasible to collect via surveys [21]. Compared to survey data, administrative data

Table 49.2 Examples of available administrative data sources in Canada for use in research related to children and youth with neurodevelopmental disorders

Organization	Data holdings	Website	Access information[a]
Canadian Institute of Health Information (CIHI)	Main gatherer and disseminator of health information in Canada	https://www.cihi.ca/en	https://www.cihi.ca/en/access-data-and-reports
Statistics Canada	Over 800 data holdings, including Vital Statistics, Discharge Abstract Database (DAD)	https://www.statcan.gc.ca/eng/start	https://www.statcan.gc.ca/eng/help/microdata
Population Data BC (PopData)	Comprehensive collections of health care, health services and population health data in British Columbia	https://www.popdata.bc.ca/	https://www.popdata.bc.ca/researchers
PolicyWise for Children & Families	Linked administrative data collected across all child and youth serving ministries in Alberta	https://policywise.com/	https://policywise.com/services/
Saskatchewan Health Quality Council	Administrative health data from the Ministry of Health and eHealth Saskatchewan	https://hqc.sk.ca/	https://www.saskhealthquality.ca/work-with-us/
Manitoba Centre for Health Policy	Comprehensive collection of administrative, registry, survey, and other data about residents of Manitoba	http://umanitoba.ca/faculties/health_sciences/medicine/units/chs/departmental_units/mchp/	http://umanitoba.ca/faculties/health_sciences/medicine/units/chs/departmental_units/mchp/resources/access.html
Institute for Clinical Evaluative Sciences (ICES)	Comprehensive collection of population health and health care delivery in Ontario	https://www.ices.on.ca/	https://www.ices.on.ca/DAS
Institut National D'Excellence en Santé et Services Sociaux	Health and social services data in Quebec	https://www.inesss.qc.ca/	https://www.inesss.qc.ca/a-propos/collaborateurs-institutionnels/reseaux-et-partenaires/deposer-une-demande/deposer-une-demande-de-projet.html
New Brunswick Institute for Research, Data and Training (NB-IRDT)	Linkable provincial administrative health and social data in New Brunswick	https://www.unb.ca/nbirdt/	https://www.unb.ca/nbirdt/data/access.html
Health Data Nova Scotia	Linkable health services and population health databases in Nova Scotia	https://medicine.dal.ca/departments/department-sites/community-health/research/hdns.html	https://medicine.dal.ca/departments/department-sites/community-health/research/hdns/services/data-access-guidelines.html
Secure Island Data Repository	Administrative health data and data from other government departments such as Education, Family and Human Services in Prince Edward Island	http://chcresearch.ca/projects/sidr/	*In progress*
Newfoundland and Labrador Centre for Health Information	Health information; the Centre is responsible for developing and implementing the province's confidential and secure electronic health record (EHR)	https://www.nlchi.nl.ca/index.php	https://www.nlchi.nl.ca/index.php/quality-information/information-requests

[a]Data access request can be a complicated process and may take a great deal of time. In some cases, researchers may have to initiate collaborations with affiliated scientists or analysts of the organization that holds the data due to lack of ability to disclose the data outside of the organization

have several advantages, including relieving response burden (data are already collected) and capturing data on individuals who may not normally respond to surveys, saving costs for data collection. However, data cleaning and analysis are more complex and time intensive. There are other important disadvantages of using administrative data to answer research questions, including atheoretical data collection and little guidance for the use of data for research purposes [30, 31]. Often, information collected is restricted (e.g., contextual information such as family information may be limited) and although longitudinal information is easy to examine, accuracy and quality of the data are always a concern [6]. For example, when examining provincial physician claims data, an increasing proportion of physician services such as mental health services are covered by other funding arrangements [32] and thus not captured, which negatively affects completeness of administrative data and ultimately interpretation of the findings based on solely administrative data. While not always complete for every research question of interest, administrative data can offer unprecedented research opportunities and help filling gaps in survey data to inform policy and practice in neurodevelopmental pediatrics.

Record Linkage

A comprehensive picture of information required for broad research applications in neurodevelopmental pediatrics can be achieved by combining different databases. Indeed, both survey and administrative databases could have potential for linkage with other databases and thus create a powerful resource for information for researchers. This process is known as *record linkage*, which identifies the same individual in different databases and allows not only to gather more information but also to assess agreement between databases. For example, using Vital Statistics Birth records linked to Hospital Separations data, it is possible to examine whether children born with very low birth weights are more likely to receive a diagnosis related to a neurodevelopmental disorder compared to children born with a normal birth weight. Since the Vital Statistics Birth database includes information about the mother, with its linkage to Hospital Separations, it is also possible to examine whether mothers of children born with a very low birth weight are more likely to have birth complications and experience longer hospital stays compared to mothers of children born with a normal birth weight. In fact, various research centers such as Healthy Child Manitoba and the Human Early Learning Partnership use a linked data approach including information not only from various administrative databases but also from cross-sectional surveys such as the CCHS. Recently, using population-based survey data from the Children's Life Style and School Performance Study (CLASS) in the province of Nova Scotia linked to administrative health data derived from the Medical Services Insurance (MSI) database and the CIHI Discharge Abstract Database (hospital separations), researchers found that poor diet quality, inadequate physical activities, and excessive use of computer and video games in childhood were associated with an increased use of health care services for attention-deficit hyperactivity disorder (ADHD) during adolescence [33]. In a similar vein, using Statistics Canada National Household Survey data linked to clinical data from the Hospital for Sick Children, researchers highlighted that lower socioeconomic status was associated with longer time to pediatric epilepsy surgery as well as poorer seizure control after epilepsy surgery, which suggested the need to address social and economic barriers for surgery [34]. Overall, record linkage has been internationally recognized as a method that maximizes the use of information to fill data gaps and to shed light on important questions relevant to neurodevelopmental pediatrics.

Research Examples

To date, secondary analyses of survey and administrative data have led to important findings related to neurodevelopmental pediatrics informing policy-relevant issues such as prevalence of neurodevelopmental disorders, health care services use and costs, as well as the necessity of family-centered care in pediatric health care. For example, using both diagnostic and functional status information from the Participation and Activity Limitation Survey (PALS), Miller et al. [9] identified 5% of Canadian children aged 5–14 years with a disability and reported that 74% of these children had neurodevelopmental disorders and disabilities (NDD/D). They also found that among children with a single type of NDD/D, 29% had a functional limitation affecting their cognitive-learning domain, 22% had a functional limitation affecting their psychological domain, and 20% affecting their social-interactive domain. Other researchers who also used data from the PALS showed that children with a severe disability were more likely to have difficulty accessing health services and to live in low-income households and in dwellings needing repairs than children who had a less severe disability [35]. Our research group, using data from the National Longitudinal Survey of Children and Youth (NLSCY), identified neurodevelopmental disorders and behavior problems and examined differences in parenting behaviors for children who had neurodevelopmental disorders with and without behavior problems as compared to children who had neither of these health problems [36]. This study also considered some social determinants of health such as child gender, parental education, and family income level, and showed that parents of children with neurodevelopmental disorders with and without behavior problems reported less positive inter-

actions with their children compared to parents of children with neither of these health problems. However, after controlling for child, parent, and family sociodemographic characteristics, only parents of children with behavior problems reported less positive interactions with their children as compared to parents of children with neither neurodevelopmental disorders nor behavior problems suggesting that parental socioeconomic status influenced these associations.

In a subsequent secondary data study, our research group investigated other social determinants of health beyond socioeconomic status and found that support from community or social service professionals was beneficial for parenting behaviors, in particular, for children with neurodevelopmental disorders [37]. Specifically, when parents of children with neurodevelopmental disorders reported receiving support from community or social service professionals, their parenting behaviors were better and did not differ from parents of children with neither neurodevelopmental disorders nor behavior problems. Other researchers who used the NLSCY focused on the prevalence of attention-deficit hyperactivity disorder (ADHD) diagnosis and prescribed medications among preschoolers and school-age children and found an upward trend during the 2000s in the prevalence of prescribed ADHD medications and diagnosis among school-age children (but not preschoolers; [38]). Overall, these studies showcase not only the potential breadth of research conducted using large-scale population-based survey data but also the benefit they can have. Specifically, the findings have important implications for research and practice in neurodevelopmental pediatrics and highlight the necessity of considering multiple factors, including social determinants of health, in understanding the lives of children with neurodevelopmental disorders.

Canada's world-class administrative-linked databases have also supported research in neurodevelopmental pediatrics. Yet, there are significant gaps in the literature related to identification of children with neurodevelopmental disorders (see [24, 39] for exceptions) in the administrative health data and understanding their quality of care, which offer opportunities for future research using administrative data. To this end, our research team attempted to apply the Children with Special Health Care Needs (CSHCN) Screener to administrative data in BC and identified 17.5% of children aged 6–10 as CSHCN [4] using linked data from PopData. Recently, using the same data, we also identified 8.3% of children with NDD/D based on diagnostic information (e.g., infantile cerebral palsy) aligned with functional status (e.g., limitations in the motor domain) [8]. These children were also more likely to live in families who received financial assistance (i.e., premium subsidy) and to receive help with the costs of BC's mandatory health care system.

In Manitoba, using linked administrative data from the Manitoba Population Research Data Repository (the Repository), other researchers estimated the lifetime prevalence of developmental disorders in children aged 0–19 years to be 2.9% based on diagnostic codes in physician claims, hospitalizations, presence of a receipt of education funding for special needs, or an assessment of Fetal Alcohol Spectrum Disorders (FASD) at the Manitoba FASD Centre [40]. They were also able to examine sociodemographic characteristics using Canadian Census survey data linked to administrative health data in the Repository and found a prevalence higher among boys than girls and among urban than rural areas [40]. In addition, in urban areas, a lower prevalence of developmental disorders was observed as income increased; yet no income gradient was observed for developmental disorders in rural areas [40]. Moreover, linked administrative data in the Repository, specifically the Medical Services and the Hospital Abstracts, Child and Family Services Applications and Intake, and the Prosecution Information and Scheduling data showed that children diagnosed with developmental disorders used more health care services including mental health services such as visits to a psychiatrist, received more social services such as being taken into the care of Child and Family Services, and were more involved with the justice system as the accused or the victim of a crime compared to children with no developmental or mental disorders [40]. Another recent study in Manitoba using linked administrative health data from the Repository indicated that among children in care, those with developmental disabilities were more likely to have a history of mood and anxiety disorders, respiratory illnesses, diabetes, hospital-based dental care, and injury-related hospitalizations as well as more ambulatory physician visits compared to those without developmental disabilities [41, 42].

In BC, using linked administrative health data, including hospitalizations, physician claims, and prescription drug data held at PopData, 8.3% of children were identified with NDD/D, and, similar to the findings in Manitoba, these children also had higher health care service utilization, including physician visits, laboratory and X-ray visits, hospitalizations, and prescription medication use compared to those without NDD/D [8].

In Ontario, using linked administrative health data housed at the Institute for Clinical Evaluative Sciences, researchers found that about 1% of children in Ontario were hospitalized children with medical complexity and 28% of these children were identified with a neurologic impairment, 12% had technology assistance, and 36% received home care services [5]. In addition, children with medical complexity accounted for almost one-third of child health spending with rehospitalization making up the largest proportion (27%) of subsequent costs [5]. In Quebec, using physician claims data, researchers found high rates of therapy changes and increased health care utilization and costs among stimulant-treated children and adolescents with attention-deficit hyperactivity disorder

(aged 6–17 years) who were prescribed atypical antipsychotic prescriptions [43].

In Alberta, using data on dispensed antipsychotic medication, diagnoses, and laboratory testing, researchers showed that antipsychotic medication use in children was associated with disruptive behavior disorders, depression, and anxiety disorders but the majority of children prescribed antipsychotic medications did not have the recommended laboratory tests [44].

These studies showcase the breadth of research and benefits in the area of neurodevelopmental pediatrics using administrative data. Nevertheless, limitations of administrative data, including incompleteness or errors in coding (e.g., incorrect or lack of entry) and underrepresentation of people with poor access to the health care system (e.g., refugees, new immigrants, people living in remote areas, Indigenous people), warrant consideration. Further work is needed to contribute to the accuracy and quality of administrative databases (see [45] for a framework) for the pediatric population, in particular, for ascertaining health conditions to advance research in neurodevelopmental pediatrics (see [46] for a scoping review). Overall, administrative data can be a useful source for conducting research or surveillance of neurodevelopmental conditions; however, additional data sources may be necessary to enhance the accuracy and reliability of the findings [24].

During the COVID-19 pandemic, one of the new data collection initiatives at Statistics Canada has been crowdsourcing. Crowdsourcing is a way to gather timely policy-relevant information. These data can be gathered quickly online from voluntary participants. However, results from crowdsourcing data collection cannot be generalized to the overall population because there is no sampling strategy. Yet, the findings may shed light on the needs of individuals and suitable support measures in a timely manner such as during and after the pandemic. One example of a crowdsourcing initiative was the data collection on *Parenting during the Pandemic*, which allowed us to examine differences in experiences between families of children with and without disabilities as reported by parents or guardians of children aged 0–14 years [47]. Relatively little has been known about the impact of the COVID-19 pandemic on families of children with disabilities. The findings indicated that while participants' various concerns for their family were similar between parents of children with and without disabilities, a higher proportion of parents of children with disabilities were very or extremely concerned for their children's amount of screen time, loneliness or isolation, general mental health, school year, and academic success. Overall, crowdsourcing initiatives can provide timely and relevant data for research opportunities. However, caution should be exercised when interpreting the results as generalizability may be limited.

Criteria to Assess a Secondary Data Source for Data Analysis in Neurodevelopmental Pediatrics

While keeping in mind the general limitations of secondary data sources, there are important criteria to consider when selecting an appropriate source of secondary data for research. These criteria include (but not limited to) the *definition* of neurodevelopmental disorders (e.g., presence of a diagnosed medical condition vs. a score on a developed scale), the *age group* included in the survey sample (e.g., young children vs. youth), the *survey design* (e.g., cross-sectional or longitudinal data), the *inclusion of a special population* (e.g., refugee, immigrant, or Indigenous children), and a *comparison group* (e.g., healthy children) as well as the *social determinants of health* that are available in the data (e.g., parental education, household income, and social support).

Researchers need to consider these criteria and to tailor questions of interest and assess the appropriateness of a secondary data source to study specific research question in topics related to neurodevelopmental pediatrics before embarking on a detailed analytical plan. For example, to address a question related to the self-esteem of children with neurodevelopmental disorders as compared with healthy children and to determine whether family income plays a role in children's self-esteem, first the definitions of the constructs of interest such as neurodevelopmental disorders, self-esteem, and family income need to be theoretically sound and fit with the available secondary data source. It is important to keep in mind that each data source necessitates a definition of neurodevelopmental disorders based on the content that is available for a specific age group included in the data source. For example, identification of neurodevelopmental disorders would be mostly based on diagnostic data in administrative health data and a proxy for child disability may be derived from enrollment in a special education class in administrative education data. In contrast, neurodevelopmental disorders can be based on reported diagnosed conditions or measures of functional status in survey data. However, national health surveys such as the CCHS are general in scope and thus may not necessarily capture child neurodevelopmental conditions specifically. For other constructs of interest, such as self-esteem and family income in our case, although the CCHS-focused content on mental health may capture information on self-esteem, other survey as well as administrative data sources may not necessarily include this measurement. Similarly, information on family income is generally included in population-based surveys but they may not be readily available in all types of administrative data sources. Thus, in some cases, the use of linked data based on record linkage may be essential to maximize the

information to fill data gaps and to shed light on important questions relevant to neurodevelopmental pediatrics.

Regarding the age group of interest, although administrative data do not usually have a restriction on age, available data may still be limited to those who have access to health care services and thus, may not capture all child and youth populations. On the other hand, national surveys may also not include children of the target age range. For example, disability-specific surveys such as the Canadian Survey on Disability (CSD; [48]) would appear to be a good fit to study neurodevelopmental disorders but it does not include national disability-specific information for children younger than 15 years of age (although disability-specific surveys, such as the older Participation and Activity Limitation Survey [PALS], did). In addition, disability-specific surveys may not include a measure of self-esteem although information on family income would be available. Child and youth surveys such as the National Longitudinal Survey of Children and Youth (NLSCY; [49]) or the Canadian Health Survey on Children and Youth (CHSCY; [50]) may appear to be most appropriate to use and provide various variables to define neurodevelopmental disorders, including parent-reported checklist of chronic conditions, measures of activity restrictions, elevated health care service utilization, and classification systems of health status such as the Health Utility Index (HUI; [13]) as well as information on self-esteem and family income. However, available data may have other limitations such as not being longitudinal or contemporary or representative of subpopulations such as Indigenous, immigrant, and refugee children and youth with neurodevelopmental disorders. Finally, with large-scale population-based databases, it is often possible to have a comparison group but not always. For example, a disability-specific survey such as the CSD would not include individuals without disabilities but may include other comparison groups such as those with different types of disabilities (e.g., physical conditions). A comparison group can be powerful to attribute findings to a specific group and increase the credibility of the findings in a given research context. For example, it is interesting to discuss the relationship of self-esteem and family income for children with neurodevelopmental disorders as compared to healthy children to better contextualize the findings (i.e., understand the differences and needs of children with neurodevelopmental disorders). However, researchers need to keep in mind that other factors may influence the findings. Therefore, comparisons between groups should be conducted after controlling for other variables that may play a potential role in the examined associations, such as social determinants of health (e.g., income and education) [51].

In summary, using secondary data sources necessitates flexibility in conceptualizing a research question and defining constructs of interest, such as neurodevelopmental disorders. In research using primary data, researchers' definitions of neurodevelopmental disorders depend on the study and researchers' purpose. In such studies, concepts, definitions, research questions, and analyses are predetermined and often fixed. With the use of secondary data, flexibility and creativity are required for concepts, definitions, and research questions, as well as how analyses should proceed with results of descriptive analyses often informing more complex analyses. Moreover, considerations of characteristics of the secondary data are important because definitions of neurodevelopmental disorders vary by age group (e.g., younger children may not be diagnosed with certain neurodevelopmental disorders until school-age) and other constructs of interest such as self-esteem may not be available.

Perhaps the best practice for a researcher is to keep in mind that there is no single data source that can provide "valid and reliable indicators of health and health care quality for children and adolescents" https://nap.nationalacademies.org/download/13084 as highlighted in a report by the Committee on Pediatric Health and Health Care Quality Measures, Institute of Medicine and National Research Council [52, p. 1]. Given this, researchers should optimize the information available in various databases by combining data from different sources. For example, children with neurodevelopmental disorders can be identified in both administrative and survey data by using diagnostic codes or special education class codes in the former and presence of chronic conditions and/or activity limitations in the latter. It is important to keep in mind that definitions need to be developed and validated to confirm that the group of interest is defined. The strengths and limitations of the construct along with the strengths and limitations of the data source need to be considered. Equally important, the agreement between administrative and survey data can be low; the reasons may be difficult to pinpoint but individual sociodemographic and health characteristics may play a role (e.g., individuals living in remote areas may have limited access to health care services and thus may be difficult to identify using administrative data-based service use information; see [53]). Overall, record linkage may provide unprecedented future opportunities for contributing to research in neurodevelopmental pediatrics.

We now encourage you to think about a research question related to children and youth with neurodevelopmental disorders, which you may wish to explore using a large-scale population-based survey data. Use the flow chart (see Fig. 49.1) to follow the steps and start using the criteria to assess a secondary data source of your choice to address your research question. For example, how will you define neurodevelopmental disorders and what age group is of interest? Is there a possibility to identify a comparison group? Where can you retrieve or access the data? Do the data meet the requirements of your research question? If not, can you modify the research question? You need to evaluate the data source, including user guides, codebooks, and tech-

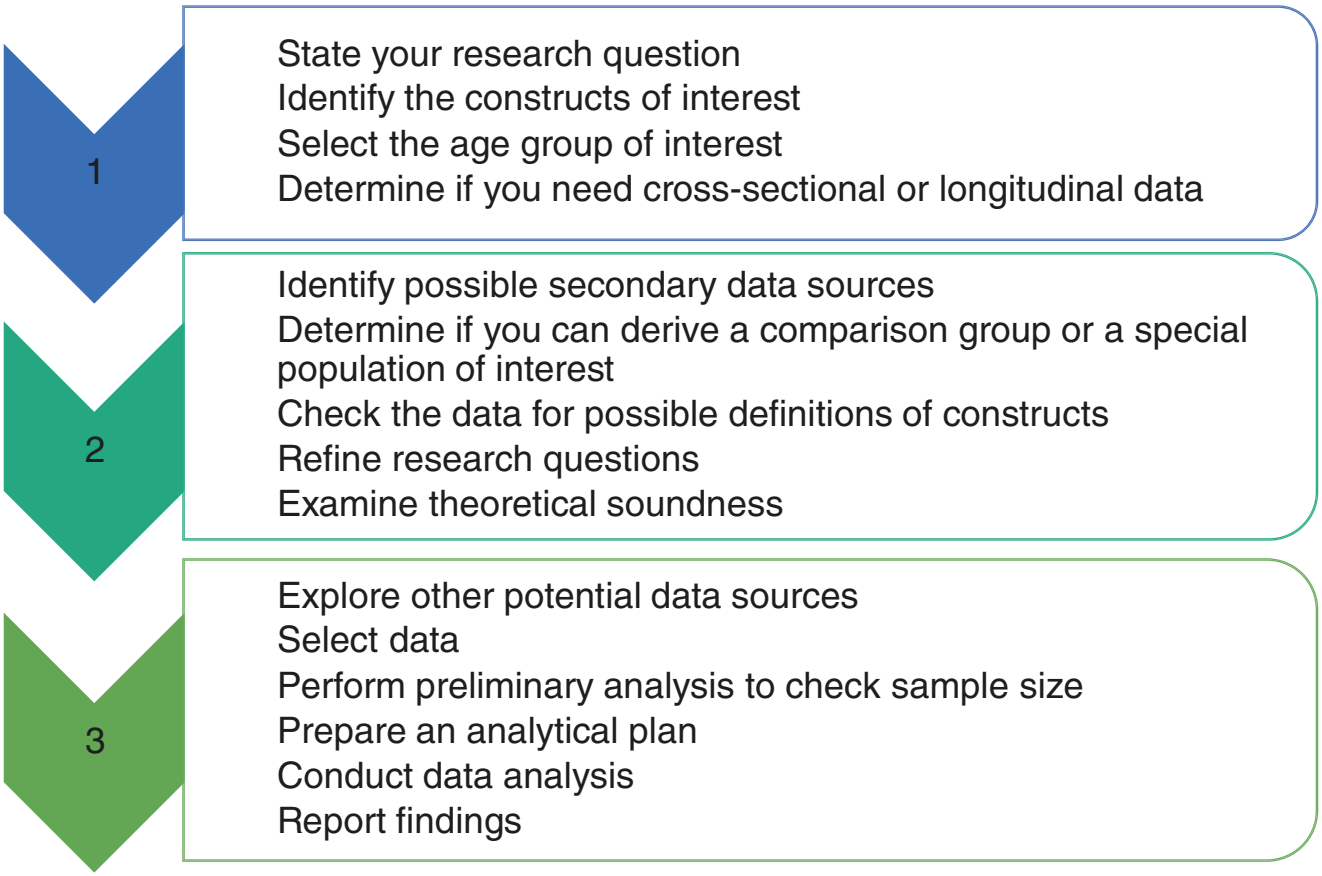

Fig. 49.1 Flowchart

nical reports. Once you determine that your evaluation is satisfactory, you can then proceed with creating an analytical plan for research.

Summary

National survey and administrative databases are two important sources of secondary data to estimate prevalence of neurodevelopmental disorders at a population level and to plan treatment and management strategies. However, both survey and administrative data have limitations. Survey data are costly and time-consuming and there are concerns about the validity of self-reported data, particularly for neurodevelopmental disorders. Moreover, surveys often only cover a limited number of neurodevelopmental disorders that are common in childhood. On the other hand, administrative data are subject to critiques about the accuracy of the diagnostic information. It is important to weigh the pros and cons of the data sources in relation to the specific research question. Often a combination of data sources may provide a more thorough understanding of issues related to neurodevelopmental pediatrics. For example, administrative data sources can quantify the number and type of visits covered by publicly funded health care services, while survey data may identify perspectives of service users from patient and provider perspectives. Overall, analyses of secondary data may provide important findings related to neurodevelopmental pediatrics informing relevant issues such as conceptualization and prevalence of neurodevelopmental disorders and policy-relevant information such as health care services use and costs as well as the necessity of family-centered care in pediatric health care.

Multiple Choice Questions and Answers (Bolded)

1. Which of the following is the main advantage of secondary data over primary data?
   (a) The secondary data are easy to retrieve
   (b) There are no fees to access secondary data
   (c) **The secondary data already exist**
   (d) There are no quality issues with secondary data
2. Which of the following is a disadvantage of population-based surveys?
   (a) They are costly
   (b) They require substantial time for data collection and development

(c) They place a burden on survey respondents
(d) **All of the above**

3. Administrative data refer to information gathered for research purposes.
   (a) True
   (b) **False**
4. Which of the following cannot be an example of administrative data?
   (a) Tax information
   (b) Health records
   (c) Criminal records
   (d) **Self-reported education information**
5. You are interested in examining a research question related to child neurodevelopmental disorders and you heard about a new population-based child survey that you might be able to use. Which of the following would not be a good practice when assessing the appropriateness of the data to your research question?
   (a) Identify the constructs of interest in your study
   (b) Check the data for possible definitions of constructs
   (c) Perform preliminary analysis to check sample size
   (d) **Conduct data analysis and report findings**

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Tools for Knowledge Dissemination and Translation to Help Your Journey from Research to Impact

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David J. Phipps and Anneliese Poetz

Learning Objectives [4, 5]

After reading this chapter, researchers and trainees will be able to:

1. Understand what impact of academic research means in terms of what funders and other stakeholders value as "impact."
2. Identify alternative frameworks for research impact and their limitations.
3. Apply the Co-Produced Pathway to Impact framework to their next "research to impact" project using the practical Research to Impact Canvas planning tool.
4. Understand the importance of stakeholder engagement and dissemination as steps toward achieving research impact using the resources provided.
5. Appreciate how straightforward research impact planning is when you have the right tools, but this does not mean you do not have to invest time and effort.
6. Identify different organizations around the world that have invested in maximizing the impacts of academic research.

Highlights [3, 4]

- You're likely already "doing" impact planning but have not had to articulate it. This chapter provides tips and tools to sketch out your research impact plan.
- There are many research impact frameworks but only one (the Co-Produced Pathway to Impact) that is anchored in stakeholder engagement and has a specific tool to help you apply it in practice.
- Ongoing and meaningful stakeholder engagement before, during, and beyond your research projects is key to achieving impact.

Introduction

The journey from research to impact is not a straight path. It meanders. It involves multiple forms of transit (i.e., stakeholders such as multidisciplinary collaborators, non-academic partners, social media networks) and these stakeholders pointing you in one direction or another. During impact planning, at best you have a sense of where you want to end up (the impact or change in the world that you hope to achieve) but not a clear pathway to get there. At worst, you hope to publish in a journal with a "high impact factor"—which is not the type of impact we are discussing. Do not get us wrong; we celebrate and support the impacts of scholarship on other scholars. Sometimes your "next user" is an academic. But that is not the end point of your research to impact journey, especially if the "end user" is a policy maker, health care practitioner, or educator.

What does impact mean, and what type of impacts are we concerned with in this chapter? According to Emerald Publishing [1], "*Impact is the provable effects of research in the real world. It is the changes we can see (demonstrate, measure, capture), beyond academia (in society, economy,*

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environment), which happen because of our research (caused by or contributed to). To create impact we need to mobilise [sic] the messages and products from our research to those who can use them. And to do this we need to engage with the wider world."

This is what this chapter can do for you and the impact of your research. In this chapter, we will help you understand what impact is, who to engage to maximize your potential to get there, and provide some tools to help you plan and execute your journey. The chapter is divided into seven sections:

1. Introduction and Background
   - Guess what, that is this section. No explanation needed (hopefully)
2. Section 1: The Co-Produced Pathway to Impact (CPPI)—*You Need to Know How to Get There from Here*
   - Description of a conceptual yet practical framework to guide your journey from research to impact, how it is different from others, and examples to illustrate how it is being applied in practice.
3. Section: 2 Research to Impact (R2I) Canvas—*You Need to Have a Plan to Get There from Here*
   - Presentation of a tool based on the CPPI, how it can help you apply the concepts in the CPPI to plan your journey, plus some examples from a recent implementation of the R2I Canvas.
4. Section 3: Stakeholder Engagement—*You Need to Know Why You Are Trying to Get There from Here and Where "There" Is*
   - Rationale for getting out of your lab/clinic and engaging with others with an interest in your work as well as a planning tool to help you plan your stakeholder engagement.
5. Section 4: Dissemination—*Necessary But Not Sufficient to Get There from Here*
   - Why doing what you think is good enough actually is not. Dissemination is necessary but not sufficient to get you to impact. Nonetheless, it is necessary, so this section will present a tool to help you plan your dissemination strategies.
6. Section 5: International Perspectives—*Others Are Also Trying to Get There*
7. The "impact agenda" is becoming a global phenomenon. This section will discuss different impact systems and make observations about assessment- vs mission-driven impact assessment. It will also present a tool to do a "health check" on how your institution is implementing impact as part of its research enterprise.
8. Summary
   - It is important to understand the practical nature of this chapter. We (the authors) are research impact practitioners. Between us we have 30 years' experience connecting science to society (or evidence to its practical application). We are informed by the research on impact, and although we have training in academic research, we are not employed as researchers. We will give you useful, practical tips and tools you can employ to guide your own journey from research to impact. What you will not get in this chapter is a deep dive into commercialization (i.e., technology transfer, patents, start-up companies). Commercialization is a practice-based antecedent of our work as it has been a common feature of academic research since the passage of the Bayh–Dole Act of 1980 [2] that influenced the US technology transfer profession. But it is not the focus of this chapter, which complements commercialization but with a focus on non-commercial impacts of research on professional (i.e., clinical, educational, social work) practice, public policy, and social services. This chapter comes at the end of the book. This is by design since the tools and practices we present can be applied to researchers at any stage of their research career in any topic covered in the preceding chapters.
   - Be prepared to map out your journey from research to impact, so you can buckle up and lean forward for the trip there.

Section 1: The Co-Produced Pathway to Impact (CPPI)—*You Need to Know How to Get There from Here*

Research impact is dominated by several popular conceptual frameworks including the Payback Model and the Knowledge to Action Cycle. The Payback Model was used as the basis for the Canadian Academy of Health Sciences (CAHS) Research Impact Assessment Framework [3], which itself was antecedent of the Alberta Innovates Health Solutions (AIHS) impact assessment framework [4]. The Knowledge to Action Cycle is not a good model for monitoring progress toward impact in an organization, since it was never meant to describe actions of a single organization and has not been described in practice [5]. Payback (and hence CAHS and AIHS) separates the research (at the beginning) from impact (at the end) and does not embed collaboration throughout the research, something that we know is key to achieving impact [6]. Neither framework creates an explicit role for the end beneficiary (i.e., the patient) as part of the process.

The Co-Produced Pathway to Impact (CPPI, Fig. 50.1) addresses some of these limitations. It comprises a five-stage model similar to CAHS/AIHS, but it embeds collaboration in the first four stages.

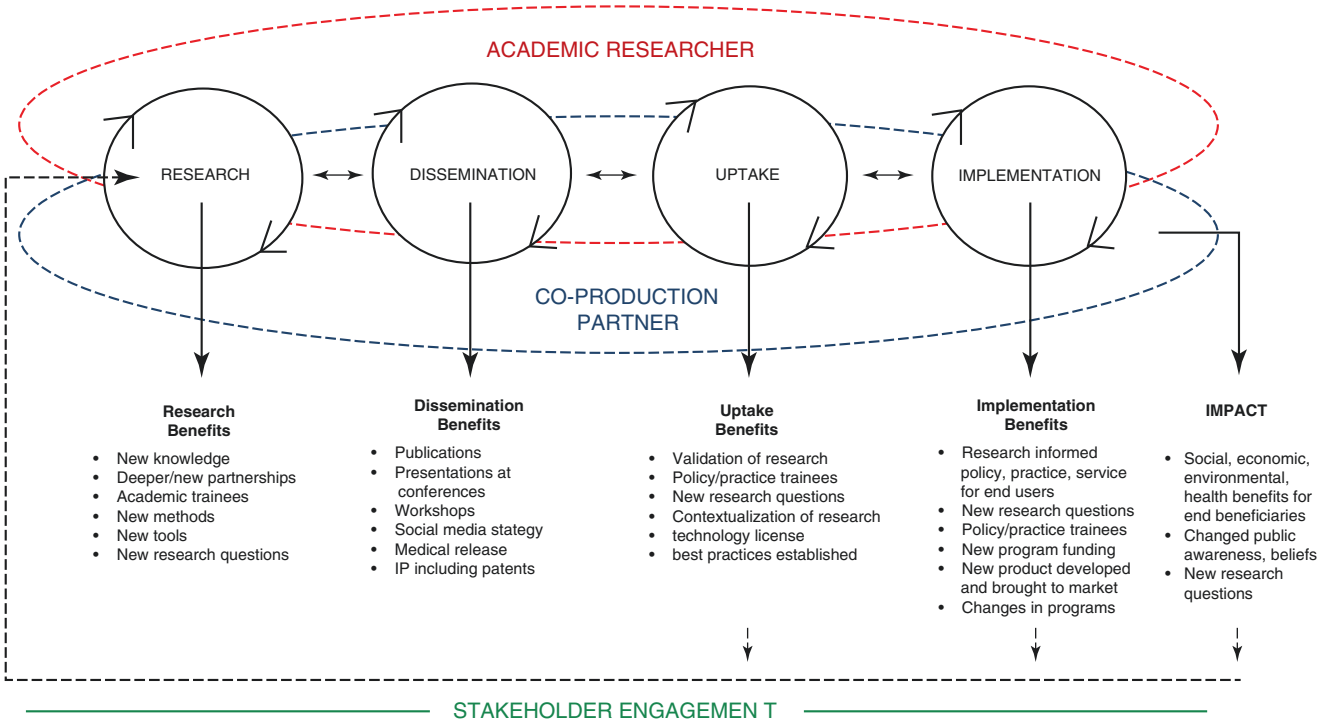

Fig. 50.1 Co-produced Pathway to Impact (CPPI)

1. **Research:** Research goals that are informed by stakeholder engagement (see Sect. 3, below) and support co-produced (=collaborative) research that maximizes the likelihood that the evidence produced is meaningful not only for academics but also for the collaborators and other stakeholders.
2. **Dissemination:** Co-produced dissemination (see Sect. 4, below) ensures that the research evidence is disseminated not only in peer reviewed literature but also in formats and in venues accessible to non-academic stakeholders, through dissemination channels they would access, and at the time they need it.
3. **Uptake:** This is the hand-off from academic to non-academic collaborators. Co-produced uptake invites the academic to facilitate the uptake of evidence in the context of its use. Facilitating the uptake of evidence in the context of its use is derived from the PARIHS (Promoting Action on Research and Implementation in Health Services) framework [7]. Do not just send your evidence to someone (=dissemination, which is not enough), participate on an expert advisory committee, or give a workshop to help potential end users understand and assess how they could use your research evidence.
4. **Implementation:** Once end users decide to use the evidence, an individual and/or organization implements it into a new public policy, professional practice, social service, etc. The organization can ask the academic researcher to collaborate on implementation including contextual adaptation.
5. **Impact:** The change(s) resulting from the implementation of the policy, practice, service, or product for those receiving the services or affected by the policy, etc. (called end beneficiaries).

The CPPI also articulates some of the benefits that could accrue at each stage of the pathway. Depending on the nature of the project, these could inform the creation of specific indicators to measure performance as the project progresses through the pathway from left to right. The CPPI can be used as a model for planning and/or evaluating policy impacts, commercial impacts, and practice impacts, but in all cases the end beneficiary (i.e., patient, consumer, student, public) helps to inform the impact(s) to strive for and their evaluation. In other words, impact is maximized by listening to and engaging stakeholders in the design of the research phase through one or more methods of stakeholder engagement (see Sect. 4, below). While the CPPI has always embedded stakeholder engagement, the Canadian Health Services and Policy Research Alliance has recently amended the CAHS framework to also include this important aspect [8].

The most significant contribution of this model can be seen in the arrow pointing to impact, which indicates that *impact is a function of non-academic partners not of researchers*. Researchers do not make products, industry partners do. Researchers do not develop public policies, government partners do. And academic researchers generally do not deliver social services, community partners do. Non-academic partners use the research evidence to

inform products, policies, and services that then have one or more impact(s) for end beneficiaries. The benefits that a class of students might gain from an experimental teaching method and the health benefits of patients in a clinical trial an be considered short-term outcomes derived from participating in the research activities. The medium and long-term impacts occur when the teaching method is deployed throughout a school board or the clinical practice being adopted as a standard of care.

Published in the *Journal of Community Engagement & Scholarship* in 2016 [9], the CPPI was applied to projects within the bullying prevention knowledge mobilization network PREVNet (www.prevnet.ca). Throughout the pathway from research to impact, the role of co-production (i.e., with non-academic stakeholders) was a major part in facilitating impact. And this is what differentiates the CPPI from other models that connect research to impact.

How Can You Use the CPPI?

As practical as the CPPI is, it only represents one generic pathway. Your project's unique pathway from genetic research to a diagnostic microarray device used by practitioners for diagnostic assessment will be different from someone else's pathway for health economics research leading to policy implementation of cost-effectiveness findings. When you are planning your research grant application, you will need to map your specific research to impact plan identifying the stakeholders and/or collaborators throughout. You also need to identify the specific indicators using the categories of "benefits" at each stage of the CPPI as a starting point. The Research to Impact Canvas (next section) will help you do this.

Section 2: Research to Impact Canvas—*You Need to Know How to Plan to Get There from Here*

The need for each research project to create a specific impact plan is illustrated by research on the UK Research Excellence Framework (REF) 2014 exercise. Jonathan Grant (Kings College, London) identified there were 3709 unique impact pathways among the 6679 REF impact case studies [10]. The experience of the REF shows that no two or three impact pathways are alike. A conceptual framework is only generic until it is specifically adapted for each research to impact project requiring impact planning tools and expertise. To effectively operationalize the CPPI for impact planning for your research, requires a tool to help you apply the generic CPPI conceptual framework to your specific plan for impact. We have created a practical tool to help you create your specific knowledge translation (KT) plan called the Research to Impact Canvas.

The KT Team within Kids Brain Health Network (KBHN) developed an impact planning tool called the "Research to Impact Canvas" or "R2I Canvas" (see Fig. 50.2). The R2I Canvas is based on the popular Business Model Canvas (BMC) used by entrepreneurs planning their start-up companies. The Business Model Canvas (BMC) is a one-page high-level business plan, characterized by nine squares organizing value proposition, product, and market. It was previously adapted to create a "triple layer" BMC that encompasses multiple types of value such as economic, environmental, and social [11]. Our adaptation of the BMC to a KT planning tool involved mapping the elements of business planning onto the elements of a KT plan, which also aligns with the CPPI. The R2I Canvas starts with the needs of stakeholders to inform goals for impact(s) as well as ongoing stakeholder engagement. This stakeholder engagement can be hands-on such as the role of a co-production partner or it can be more informative with end users testing and providing input on the ongoing design and development of KT or commercial products. Table 50.1 describes the different components of the R2I Canvas in detail. You can use the R2I Canvas to create a high level Knowledge Translation plan which can be used as an outline for writing a more fulsome plan into your grant applications. This plan also helps you to determine the benefits that accrue for stakeholders at different stages of the CPPI including impacts, which can inform your associated impact evaluation plan. Since the R2I is based on business principles, you can also use it to inform conversations with potential partner(s) because it will help you to articulate the value proposition(s) of your work for specific stakeholder groups, or, target audiences.

For Kids Brain Health Network, we used the R2I Canvas to support the development of impact strategies for research grant applicants. In the 2017 "Request for Applications" (RFA) funding competition (due date October 7, 2017), Kids Brain Health Network sought to fund the best projects, defined as those that proposed solutions to address identified stakeholder needs published in KBHN's recent environmental scanning report (see Sect. 4, below). KBHN aims to achieve impact(s) for its stakeholders in the form of improved policy, practice, and quality of life for children and families affected by neurodevelopmental conditions. By providing the opportunity for applicants to obtain assistance with their KT plans, KBHN aimed to maximize the chances that these applications would become successfully funded due to being planned for achieving real impact(s) for its stakeholders. It would also enable evaluation and progress reporting.

To support RFA applicants using the R2I Canvas, the KBHN KT team developed a short overview video (https://www.youtube.com/watch?v=Cz0l1viEFmc&t=5s) that explained how to use the Canvas. A second longer presentation was given as a webinar for all applicants, which included a demonstration using a fictitious example. In addition, RFA applicants had the opportunity to receive one-on-one KT support. From the perspective of researchers, it was this one-on-one support that was the most effective for applying the R2I

Project Title: _____ Funding Start Date: _____ Funding End Date: _____

What is the stakeholder need you will be addressing: _____

IMPACT desired (what changes you are hoping to achieve/contribute toward achieving): _____

Stakeholder(s) (Target Audiences, Customers or End Users)	Stakeholder Engagement	Benefit of the RESEARCH for Stakeholders	Key Project Activities & Outputs	Key CO-PRODUCTION partner(s)
	Networks		Key Resources Needed to Deliver the Benefits for Stakeholders	
UPTAKE and IMPLEMENTATION			Budget for doing the project & KT & Commercialization activities	

Fig. 50.2 Research to Impact Canvas

Canvas and increasing their understanding about both the simplicity and complexity of impact planning.

After the closing date for applications we conducted an open-ended survey with all applicants who utilized the available KT services ($n = 17$). Question one, with three separate open-ended text boxes (1a, 1b, and 1c) asked: *When it comes to the support you received from the KT Team for KT planning (including the use of the R2I Canvas) what is the single biggest difference it has made?* Answers ($n = 12/17$ or 71% response rate) were coded as negative, neutral, or enthusiastic and are presented in Table 50.2. Most respondents were enthusiastic about their experience using the R2I Canvas.

We also asked researchers a multiple-choice question: *Based on your experience with the R2I Canvas, would you use it again?* The majority (8/12 or 67%) indicated they would "definitely use it again" with nobody (0/12) responding they would "definitely not use it."

Testimonials received about the tool included:

- *Confidence the tool creates*: "I would say that I'm much more confident in knowing what activities contribute to effective KT."
- *Future use of the tool for other grant applications*: "I will now incorporate this canvas into other upcoming grant applications because it is so user-friendly."
- *The tool itself*: "Provides a high-level overview of the details to follow in the application. Helps to clarify and focus aims/objectives, and makes you think about how to actually execute the goals of the project."

We have created a variety of downloadable version of the R2I Canvas for you to use. Three versions are available for download, including:

1. Blank Canvas with fillable sections: https://www.slideshare.net/NeuroDevNet/researchtoimpact-canvas-one-page-planning-tool-for-research-kt-and-commercialization-85874316?from_action=save
2. Canvas editable with instructions: https://www.slideshare.net/NeuroDevNet/researchtoimpact-canvas-editable-with-instructions-one-page-planning-tool-for-research-kt-and-commercialization-85874215
3. Canvas with instructions only: https://www.slideshare.net/NeuroDevNet/research-toimpactcanvas-instructionsreferenceonly-not-editable2017july6kbhn-85874087

How Can You Use the R2I Canvas?

The R2I Canvas can help you turn the CPPI conceptual framework into a specific pathway for your research to impact project. Once you have completed the Canvas you will have all the information you need to write your impact strategy (or KT plan) in your grant application. You can illustrate your specific pathway to impact based on the CPPI and then describe the elements of the Canvas in the text of the grant application. You can even use the Canvas as an appendix if the grant program allows attachments.

Table 50.1 Elements of the Research to Impact Canvas

R2I Canvas component	Description
Impact(s)	Before you begin planning your project, how do you know it is relevant or needed? Stakeholder engagement with your intended end users will help you identify the short-term, medium-term, and long-term impact(s) you need to plan your project around toward achieving them
Stakeholders	Target audiences, end users, end beneficiaries, customers • Who are most critical for you? • Identify the major categories (e.g., physicians) and what are the sub-categories (e.g., sub-disciplines in medicine such as developmental pediatricians) • Do you have access to them? If not, how will you gain access? • Are there any missing?
Stakeholder engagement	How do you intend to interact with your different stakeholder groups and for what purpose? How are they integrated into research and evaluation activities? How will they inform the design and development of your research questions, KT, and commercial products?
Networks	Networks/channels for dissemination What are your end users' preferences for receiving information and/or purchasing products? How are you currently reaching stakeholders? How can your contacts/stakeholders help disseminate through their existing networks?
Benefits (=value) to stakeholders	What is the value proposition for each stakeholder group you identified in the first row of this table?
Project activities	What activities will be required to progress from research to impact? Consider: 1. Co-production of research, KT products, and/or commercial products with stakeholders 2. Dissemination (including commercialization) of research evidence and KT products and/or marketing of commercial products to stakeholders 3. Facilitated uptake with stakeholders 4. Evaluation of impact(s)
(Co-production) Partners	A type of stakeholder, co-production partners are those partners who will work alongside academic researchers contributing expertise, cash, and in-kind resources to the project
Key resources	What non-financial resources (human, data, space, equipment, software, etc.) will you need to complete the project activities? Identify any resources you do not have and a plan to secure these
Uptake and implementation	Describe how you intend the research should be taken up by end users (see facilitated uptake, above) and what efforts are needed by whom to help end users implement research outputs into policies, products, practices, and services. How will your networks help move your products into use?
Budget	How much money do you need to complete your project's activities and acquire the key resources you need? Remember to budget for impact assessment (evaluation) including collecting the evidence of engagement (usually via interviews) and data to be collected and analyzed throughout the research to impact process

Table 50.2 Results of R2I Canvas open-ended survey questions

Question: What is the single biggest difference it has made…		Answers
1a	…for your recent KBHN RFA application?	8 enthusiastic 3 neutral 1 negative
1b	…for your (ability to apply for) future funding?	8 enthusiastic 4 neutral 0 negative
1c	…for your confidence in being able to do KT?	8 enthusiastic 4 neutral 0 negative

Section 3: Stakeholder Engagement—*You Need to Know Why You Are Trying to Get There from Here and Where "There" Is*

The AccountAbility 1000 Stakeholder Engagement Standard [12] defines stakeholders as "…those groups who affect and/or could be affected by an organization's activities, products or services and associated performance" (p. 5). There are different types of stakeholders, defined by their: professional roles (e.g., physician, educator, interventionist, etc.), relationship to the issue at hand (e.g., self-advocate, policy maker, frontline worker, patient/client, parent or caregiver, family member, etc.), and/or relationship to the project and its outputs (co-production partner, collaborator, co-designer, prototype-tester, informant, advisor, recipient/user of the outputs). Therefore, different stakeholders will inevitably play different roles at different stages throughout your research to impact pathway. There are also different levels of power (i.e., authority) within stakeholder engagement. Arnstein's ladder of citizen participation is a seminal paper outlining different levels of power provided to stakeholders to participate, from tokenistic engagement to stakeholder-led engagement [13]. The most important thing to know about stakeholder engagement is this: if you do not really want to engage stakeholders but you are doing it because your funder requires it, that is tokenism. "Stakeholder engagement is the

process used by an organisation to engage relevant stakeholders for a clear purpose to achieve agreed outcomes. It is now also recognised as a fundamental accountability mechanism, since it obliges an organisation to involve stakeholders in identifying, understanding and responding to sustainability issues and concerns, and to report, explain and answer to stakeholders for decisions, actions and performance" [12] (p. 5).

Engaging stakeholders throughout the research to impact journey will increase your confidence that the research you are conducting meets not only your academic curiosity but is also meeting an unmet need for a parent, a policy maker, a clinician, or an educator. Bowen and Graham articulated the failure to bridge the knowledge to action gap (i.e., the failure to achieve research impact) not as a failure of knowledge transfer but a failure of knowledge production [14]. If you are not creating value by working on something that end users need, they will never use the research evidence, KT, or commercial products. This is why the R2I Canvas is based on the BMC, because in business it is common sense that if you are not creating value for your customers they will not buy your product. So get out of your labs, clinics, and studios and find out what your intended target audiences need. What are the methods you can use to authentically engage stakeholders?

Kids Brain Health Network KT team searched for, vetted, and summarized a number of stakeholder engagement guides and developed tools to help you engage with stakeholders. The Stakeholder Engagement Guide [15] begins with a distillation of many methodologies into three tables: to tell, to tell and listen, and to listen. The planning tables in appendix B of this guide will help you think through more detailed elements including:

- Type of stakeholder, i.e., parent, teacher, physician, etc.
- Key organizations related to different types of stakeholders, i.e., health advocacy organization, college of teachers, teachers' union, college of physicians, etc. to help you recruit stakeholders for engagement.
- Role of each key organization, i.e., provide referrals, provide testimonials of lived experience, provide data.
- Desired level of participation: see Arnstein's ladder of engagement (above).
- Method for engaging: choose from Tables 50.1, 50.2, and 50.3 in the Stakeholder Engagement Guide for activities that allow you to speak to stakeholders (Table 50.1), speak and listen to stakeholders (Table 50.2), and listen to stakeholders (Table 50.3).
- Articulate how stakeholders will benefit from the engagement. What is in it for them?

The KBHN KT team identified stakeholder priorities for future research funding by designing a mixed-methods stakeholder engagement approach. During 2016–2017, we conducted a qualitative (grounded theory) study using telephone interviews (ranging in length from 45 min to 3 h) with 32 stakeholders (policy makers, practitioners, and parents). Using qualitative analysis (open and axial coding), these data allowed the identification of 44 stakeholder needs. We then needed to ask stakeholders to help us prioritize these, since we could not do all 44 at once. At an in-person event held in January 2017, we asked the 25 attendees to vote and then discuss in detail, their top 10. While this consultation provided detailed wisdom from stakeholders about how KBHN could begin to address them, we recognized that 25 votes from the Vancouver region did not provide us with the pan-Canadian representation KBHN needed for strategic planning. We adapted the in-person voting process, to an online survey format, which allowed us to obtain a much larger and more representative sample. After cleaning the data, there were 656 analyzable responses. The top six stakeholder needs that are within the KBHN mandate to address, are:

1. Specialized in-depth training for educators (533 votes).
2. Reduction/removal of barriers to diagnosis (446 votes).
3. More professionals working in the school systems such as occupational therapists, physical therapists, speech language therapists (410 votes).
4. Non-categorical treatment for functional deficits (368 votes).

Table 50.3 Key elements of a dissemination plan

Goals	What do you hope your dissemination activities will accomplish in the short term (<12 months) and long term (>12 months)?
Who	Who and where are your stakeholders (you will have done this in your overall impact plan, and more specifically in your stakeholder engagement planning in Sect. 4). What type of stakeholder (i.e., clinicians), in what organizations will you find them (i.e., hospitals), and what networks can you use to reach out to them (i.e., Canadian Association of Paediatric Health Centres)?
How	How will you connect with your stakeholders to find out the channels they use to receive information? What type of knowledge products do they need?
Key messages	What do your audiences tell you they need to know? What else do you want to tell them?
When	When is the best time for sharing your key messages?
Activities	Informed by "how" (above), what combination of activities/products/events will you undertake to achieve the dissemination goals?
Expertise/ skills	What skills do you/your team need, to undertake these activities? Who will be responsible for which activities?
Barriers/ enablers	Identify any circumstances, individuals, etc. that could help you achieve your dissemination goals (facilitators) or that could delay or impede your success (barriers)
Evaluation	How will you evaluate if you have accomplished your short- and long-term goals? What data (qualitative and quantitative) sources will you use, and do you have access to those data?

5. Early diagnosis for targeted supports (364 votes).
6. More resources and more efficient use of existing resources (348 votes).

The methods and results within this modified grounded theory approach resulted in the identification of the needs are presented in a Stakeholder Engagement Report [16] and the quantitative ranking of those needs from the online prioritization survey is presented in an addendum.

KBHN has used these top six priorities to increase the relevance of its work by requiring that applicants in the 2017 Request for Applications identify how their research will address one or more of the identified priorities. Funding research that meets identified needs will maximize the chances that research outputs will be relevant to end users and end beneficiaries enabling uptake of the disseminated research evidence (see Sect. 5, next).

How can you use Stakeholder Engagement?
If you are interested in (or required to consider) how your research might have an impact beyond your publications, before you craft your grant application you have to understand the needs of your stakeholders. Use one or more of the methods presented in the Stakeholder Engagement Guide above, along with the form-fillable appendix to plan your stakeholder engagement. Creating research goals that are meaningful for you and your stakeholders maximizes the potential for your work to mature to impact, as illustrated by the CPPI. But beyond a single reach out to stakeholders at the beginning, plan to stay in regular contact with stakeholders. Reasons to connect with stakeholders include: updates as research results emerge, asking for ongoing input as the research progresses and as you develop KT and/or commercial products, distributing information on upcoming events, disseminating information and/or KT products, helping to facilitate the uptake of evidence in their context, and informal updates on how their input has been or is being used, etc. You may choose to form a stakeholder advisory committee. You might have a stakeholder Linked In group to which you can post information. However, you stay in touch make sure it includes a two-way flow of information.

Section 4: Dissemination—*Necessary But Not Sufficient to Get There from Here*

Eventually, you will need to disseminate your research findings to academic and non-academic stakeholders. As mentioned earlier, dissemination is necessary but not sufficient to catalyze change. However, dissemination is still the most commonly described knowledge mobilization activity and it is necessary.

Dissemination includes making research accessible and understandable [17]. Dissemination may occur in the context of discussion with experts, where being able to ask and receive answers to questions that are specific to a particular context or situation can increase understanding that can then become applied. While dissemination is not enough for achieving impact, it is one of the necessary steps along the path because implementation cannot occur unless you have something to implement.

In order to maximize the reach of your findings you will need a dissemination plan. Similar to the Stakeholder Engagement Guide, the KBHN KT Team developed a Dissemination Guide [18], which starts out with an annotated bibliography of open access dissemination guides and ends with an appendix of form-fillable worksheets to help you create your dissemination plan.

Each worksheet in the Dissemination Plan Guide of Guides covers the key elements of your dissemination plan. These key elements are presented below in Table 50.3.

Some interesting observations from this dissemination planning tool:

1. Your actual dissemination activities are not described until step 6 of 9. Many researchers start with dissemination activities (i.e., writing a clear language research summary, posting on a web page, etc.) before considering if the relevant audiences actually want the information in that format. Similar to impact planning, understanding your audiences and what they need is key for maximizing uptake of the knowledge you share.
2. Timing is an important consideration. Knowledge translation is, getting the right information to the right people in the right format at the right time to inform decisions and/or best practice. We often forget to consider timing. If your audience includes parents, then do not hold an information session during March break when they might be on vacation. If your audience includes parliamentarians, consider reviewing the leg-

islative calendar so you can anticipate when you might be able to get their attention. If the audience is policy makers, find out when they will be re-evaluating their policies; most levels of government review their policies on a three-year cycle.

3. Speaking (and listening) to your stakeholders is key to find out where they go for their information. There is no point in undertaking a twitter strategy when your stakeholders are on LinkedIn.

How Can You Use Dissemination?

We often read impact strategies that start and end with dissemination, but this approach is incomplete. In addition to co-production and stakeholder engagement, moving your research findings toward application requires that you disseminate to academic and non-academic audiences. Use the Dissemination Guide to plan your dissemination strategy. Use insights gained through your stakeholder engagement activities for multiple purposes: to inform your impact plan and to identify dissemination activities you can pursue as part of that overall impact plan.

Section 5: International Perspectives—*Others Are Also Trying to Get There*

When you look around the world there are lots of examples of investments in research that are seeking to maximize the impacts of research by supporting knowledge mobilization/translation activities. KNAER (https://www.knaer-recrae.ca/) is an excellent example of an organization that connects research evidence to its use by non-academic partners such as schools, school boards, the Ministry of Education, and non-profit organizations. The Canadian Partnerships Against Cancer (https://www.partnershipagainstcancer.ca/) is a national knowledge mobilization organization seeking to ensure that research evidence across the cancer spectrum (prevention, screening, treatment, and palliative care) is applied across the cancer care system including federal, provincial, and non-profit cancer organizations. Other examples of organizations investing in maximizing the impacts of research include: Gambling Research Exchange Ontario (http://www.greo.ca/en/index.aspx), Ontario Institute for Work and Health (https://www.iwh.on.ca/), and Parachute Canada (http://www.parachutecanada.org/). There are many other Canadian and international examples. Beyond individual Canadian organizations, there are national systems of research impact (see Fig. 50.3).

Fig. 50.3 Some international impact systems

- REF is the UK Research Excellence Framework (www.ref.ac.uk). It is a system-wide impact assessment exercise that introduced impact into the 2014 exercise and will be repeated in 2021. In addition to impact (25% of the total REF score), REF includes an assessment of research excellence and of the institutional research context.
- The SEP is the Standard Evaluation Protocol (https://www.knaw.nl/nl/actueel/publicaties/standard-evaluation-protocol-2015-2021) in the Netherlands. It is an institutional self-assessment system where institutions assess research quality as well as impact.
- The Australian Research Council conducts not only the Excellence in Research Australia (https://www.arc.gov.au/excellence-research-australia) assessment but also has moved to include the Engagement and Impact pilot into a system-wide Engagement and Impact Assessment (https://www.arc.gov.au/engagement-and-impact-assessment).
- While impact is an optional element of New Zealand's (NZ's) Performance Based Research Fund (http://www.tec.govt.nz/funding/funding-and-performance/funding/fund-finder/performance-based-research-fund/), researchers can describe the impacts their research has made while also articulating the scholarly impacts of their research.
- Development Research Uptake for Sub Saharan Africa (www.drussa.net) is a collaboration among 24 African universities investing in what they call "research uptake" (=knowledge translation) so that university research could benefit local communities. It was funded by overseas development funding from UK DfID (https://www.gov.uk/government/organisations/department-for-international-development) and managed by the Association of Commonwealth Universities (www.acu.ac.uk).
- The National Alliance for Broader Impacts (www.broaderimpacts.net) is funded by the U.S. National Science Foundation (NSF) to support the development of research impacts. The U. Missouri Connector (http://theconnector.missouri.edu/) has expanded beyond NSF grants in science, teachnology, engineering and math (STEM) disciplines to support connections between researchers and society in all disciplines.
- And finally, back home in Canada, while we have many examples like KNAER (above), we also have a national research impact network, Research Impact Canada (www.researchimpact.ca), a network of 17 universities investing in supports and services to create the conditions for impact.

That is a nice, international list but so what?

What is interesting are the drivers behind each national system. The research impact systems in the UK, Netherlands, NZ, and Australia are assessment driven. Universities are required to articulate the impact that researchers are making, which for the UK and NZ drives additional institutional funding. This creates incentives but also pressures the potential for gaming the impact system [19].

For universities in Canada, the USA, and Africa, the impact "agenda" is mission driven, not assessment driven. In these countries, universities are creating the conditions for research impact because it aligns with the mission of these institutions not because they are required to do so by a top-down, government-driven directive.

What does this mean? In Canada it means we focus more on how to create impact and less on articulating what impacts have occurred. In Canada it means that fundamental research can take place with only scholarly impact as the intended goal. It means that for disciplines like cosmology, mathematics, philosophy, etc., research can be conducted with a view to discovering and communicating new knowledge, without needing to create impacts beyond scholarship. It also means that for researchers within certain disciplines that might be more likely to achieve impact (like education research, nursing research, etc.), their universities are stepping up to help. But are universities supporting the creation of the conditions for impact in ways that also impose burdens and obligations for achieving it?

The Institutional Healthcheck Workbook [20] is based on research impact literacy [21] focusing on the institution and not the researcher/project. Ultimately, the researcher/project is the beneficiary of institutional impact supports and services, but the Healthcheck Workbook can help identify strengths and gaps in an institution's supports for impact along five elements:

1. **Commitment:** The extent to which the organization is committed to impact through strategy, systems, staff development, and integrating impact into research and education processes.
2. **Connectivity:** The extent to which the organizational units work together, how they connect to an overall strategy, and how cohesive these relationships are.
3. **Clarity:** How clearly staff within the institution understand: impact, how impact extends beyond traditional expectations of academic research, and their role in delivering impact.
4. **Competencies:** The impact-related skills and expertise within the institution, development of those skills across individuals and teams, and value placed on impact-related specialists.
5. **Co-production:** The extent and quality of engagement with non-academics to generate impactful research and meaningful effects.

The Institutional Healthcheck workbook provides a scoring rubric under each of these five elements that allows the user to create a spider plot to identify any institutional gaps.

The institution can then develop interventions to build capacity to address them. Using the scoring rubric at different time points allows the institution to track progress toward its impact goals.

How Can You Use International Perspectives?
There is a lot of work underway in different countries that can be relevant to your work in your country on your project. There is a literature and evidence base underpinning impact-related practice that is transferable to a variety of contexts. Just as you base your research on building upon the literature that has preceded yours, it is important to base your impact work on the global literature and evidence that has preceded your efforts at achieving research impact. You can do this by using the established tools presented in this chapter or by hiring a KT specialist with these skills to help you. Don't feel you need to invent your own tools or that you're on your own.

Conclusion

So far, this chapter has described: what pathways to impact are, both generic and specific to your projects; planning for impact; stakeholder engagement; dissemination; and, international perspectives on research impact. But two questions remain:

1. So what? We do this work not only to create new knowledge but also with the hope that new knowledge will ultimately have an impact, in the context of KBHN for children and families living with a neurodevelopmental condition. No one writes a grant application, even the most basic biomedical grant application, without first understanding the potential benefits to children and families. Plan your impact strategy in a way that includes stakeholder engagement, and dissemination to continue your engagement with these stakeholders helps create the conditions for impacts arising from new knowledge gained from your research.
2. Now What? You can use the tools presented in this chapter when writing your next grant application, but give yourself enough time to meaningfully engage stakeholders. You can also seek help from research impact practitioners such as those in networks such as Kids Brain Health Network (www.kidsbrainhealth.ca) and Research Impact Canada (www.researchimpact.ca) member universities.

Impact planning is not a burden or "one more thing" you must do. Impact is something you are already striving toward, and you have likely been planning for impact in informal ways up to now. The tools for knowledge dissemination and translation described in this chapter will help you articulate your impact plan, a requirement of most funders, and can help you do it more effectively.

Multiple Choice Questions [4, 5] [correct answer in bold]

1. What are some conceptual frameworks in the field of research impact?
   a. **The Co-Produced Pathway to Impact, the Payback Model, the Knowledge to Action Cycle**
   b. The Co-Produced Journey to impact, the Payback Model, the Knowledge to Action Cycle
   c. The Payback Model, the Knowledge to Action Cycle, the Research to Impact Canvas
   d. None of the above
2. Please select the answer that is true about these impact planning frameworks:
   a. KTA was never meant to be and never has been described as applied in practice
   b. Payback does not incorporate stakeholder collaboration throughout the research
   c. Neither KTA nor Payback creates an explicit role for the end beneficiaries of the research
   d. The Co-Produced Pathway to Impact is a 5-stage model that incorporates stakeholder engagement
   e. **All of the above**
3. The Research to Impact Canvas is a practical tool based on the Business Model Canvas, which is intended to help researchers apply the Co-Produced Pathway to Impact planning for their own research projects (**True**/False)
4. What are the drivers behind the national systems for investment in research impact?
   a. Assessment
   b. Mission
   c. Funding
   d. Allocation
   e. **a and b only**
   f. All of the above
5. What are the five elements in the Healthcheck Workbook that help identify strengths and gaps in an institution's support for impact?
   a. Commitment, Connectivity, Charity, Competencies, Co-production
   b. Commitment, Constancy, Charity, Competencies, Co-production
   c. **Commitment, Connectivity, Clarity, Competencies, Co-production**
   d. Commitment, Connectivity, Clarity, Competencies, Co-produced Pathway to Impact
   e. None of the above

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