Manuel F. Casanova · Ayman S. El-Baz
Jasjit S. Suri *Editors*

Imaging the Brain in Autism

Springer

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I dedicate this book to my four daughters who through their love have made my life enjoyable. I would also like to acknowledge my grandson Bertrand Might as the person who has motivated my pursuit for doing research in autism.

Manuel F. Casanova

I would like to dedicate this book to my wife, daughter, son, mother, and father.

Ayman S. El-Baz

I dedicate this book to my family and to Behavioral Imaging (http://www.behaviorimaging.com).

Jasjit S. Suri

Preface

Data compiled by the Centers for Disease Control and Prevention indicates an alarming and continuing increase in the prevalence of autism. According to the latest survey, among 14 different sites on the Autism and Developmental Disabilities Monitoring (ADDM) Network, the estimated prevalence for autism spectrum disorders (DSM IV-TR) was 11.3 per 1,000 (one in 88) children aged 8 years of age who were living within these communities during 2008. This translates to approximately one in 54 boys and one in 252 girls living in the ADDM Network, a 78 % increase compared to the prevalence reported one decade ago.

More alarming statistics are derived from other countries where the prevalence for autism has been reported as higher than in the USA. In South Korea, for example, the prevalence is 1 in 38 children. The latter study, which screened an overwhelming 55,000 12-year-old children, suggests that when canvassing an entire population, rather than sampling the same, prevalence rates may be higher than expected. Still, it could be argued that higher prevalence rates may be the result of detecting other developmental conditions along with autism.

Along with the rising prevalence rates, there is a new research that estimate autism costs society a staggering $126 billion per year within the USA alone. The number has more than tripled since 2006. Nonmedical costs such as special education and child day care account for the greatest proportion of expenses with the majority of costs being incurred during adulthood due to residential care and loss of productivity. Cost varies according to the communities the patients live in and the local government agencies that provide education, welfare benefits, and housing. Still, the latest figures do not capture the full impact of the condition for which other financial burdens have still to be considered. The primary caregiver (typically the mother) who has to serve as case manager and advocate for her children usually is negatively impacted in her earning potential. Mothers of children with autism spectrum disorders are thus more likely to work fewer hours and earn less than mothers of neurotypicals or those with other health limitations.

Despite intensive research during the last few decades, autism remains a behavioral-defined syndrome wherein diagnostic criteria lack in construct validity. In essence, contrary to other conditions like diabetes and hypertension, there are no

biomarkers for autism. Diagnosis by behavioral assessments usually occurs around the age of 2 or 3. New imaging methods are changing the way we think about autism, bringing us closer to a falsifiable definition for the condition, identifying affected individuals earlier in life, and recognizing different subtypes of autism.

Early recognition of signs of autism is important as it can lead to early intervention. Controlled studies show that early behavioral-based interventions change the outcome of affected children by significantly improving IQ, cognitive and language abilities, as well as adaptive behaviors. The American Academy of Pediatrics now recommends that children get screened for autism during regular checkups at ages 18 and 24 months. Unfortunately, developmental and behavioral screening tools lack sensitivity to screen specifically for autism and usually require follow-up with an autism screening tool when developmental concerns arise. Even then, autism screening tools have not been widely validated under 18 months of age. In this regard it is useful to consider these instruments as useful guides to inform individuals as to the potential risk for autism rather than regard them as diagnostic tests.

Autism is a challenging condition that has intrigued both clinicians and researchers alike because of its association with significant cognitive disturbances in the absence of gross brain abnormalities. Initial findings suggest that the pathology of autism is at a higher level of resolution, one that may escape gross visual inspection. Unfortunately, few neuropathological studies have been reported in autism, in part, due to the limited availability of postmortem tissue. Early studies suggested the presence of cerebellar pathology in autism. More recently studies have elaborated on abnormalities related to cortical thickness, lamination, and migrational disturbances.

The use of and focus on the cerebral cortex provides a paradigm shift in our approach to this condition. Early studies suggested that the cerebellum was the only site by both imaging and postmortem data wherein cell loss was reported by multiple independent laboratories. More recent imaging studies have shown that MR images of the cerebellum in a substantial number of patients are indistinguishable from those of control subjects. Furthermore, the presence of reactive gliosis to Purkinje cell loss suggests an acquired process. A recent immunocytochemical study comparing cell counts between Nissl-stained sections and calbindin indicates that possible agonal circumstances and even postmortem handling could have accounted for lower Purkinje cell counts in earlier studies. It may be that in many cases, Purkinje cell loss may be related to seizures, medications, and agonal events rather than to a core pathology of the condition.

It is unsurprising that neurologists consider autism a disease of the cortex rather than the cerebellum. The presence of seizures in a significant proportion of cases along with the absence of spasticity or vision loss supports this tentative localization. Furthermore, dysfunction of multiple higher cognitive functions indicates a widely distributed defect involving the cerebral cortex.

The brain's capacity to perform cognitive tasks is made possible by the proper association in function of different brain areas arranged as networks. Research on brain connectivity not only has important implications into the pathophysiology of autism but also presents opportunities/targets for intervention. These promising

findings can provide the first steps towards developing a biomarker that could complement or add construct validity to our present diagnostic criteria. Ultimately imaging techniques may distinguish autism from other developmental conditions, even those that share common symptoms such as speech delay or attention deficits.

Structural brain imaging in conjunction with machine learning methods based on criteria such as cortical thickness is able to classify individuals in the autism spectrum with as much as 90 % accuracy. Other studies using diffusion tensor imaging (DTI) have shown asymmetries between the hemispheres that bear on so-called hot spots associated with motor skills, attention, facial recognition, and social-functioning behaviors that are abnormal in autism. In essence the two hemispheres must work together when performing many brain functions and research is capable of identifying the strength of these connections in autistic patients.

Imaging of structural changes has found striking differences starting at 6 months of age in high-risk infants who later develop autism. The findings are in keeping with autism being a neurodevelopmental condition, one that does not appear suddenly but has its roots in brain development. Furthermore, findings implicate multiple fiber tracts suggesting that autism is a whole brain phenomenon.

It is our hope that in the future some of these structural modalities will be correlated to electrophysiological methods. The blueprint of connectivity for the brain in autism may relate to how we create conscious perception. Gamma frequencies in particular seemingly bring together a distributed matrix of cognitive processes into a coherent cognitive act. A common trait among autistic individuals is that they can see the trees but not the forest. They become wrapped up in details but miss the larger picture. This translates to socialization deficits, which are dependent on integrative concepts as opposed to isolated ideas or sensory inputs.

All of the imaging modalities that we have discussed are important pieces within the autism puzzle. They emphasize the power of new technology to uncover important clues about the condition and give hope for developing effective interventions. This book was created to examine autism from this unique perspective: one that would emphasize results from different imaging technologies. These techniques do show brain abnormalities in a significant percentage of patients, abnormalities that translate into aberrant functioning and significant clinical symptomatology. It is our hope that this newly found understanding will make the field work collaboratively and to provide a road that minimizes technical impediments.

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Chapter 1
Introduction to Neuropathology

Manuel F. Casanova, Paul H. Patterson, and Eric London

1.1 Introduction

Tissue and cell activity occurs within a spectrum spanning both health and disease. These changes are reflected in the structure of different anatomical elements. Fortunately, there is only a limited number of tissue and cellular changes that, because of their reproducibility, can be used for diagnostic purposes by a trained physician. These changes are the essential morphological features that I will expand upon in the next couple of sections. I will first describe these changes at the cellular level before tackling a higher level of complexity in terms of tissue reactions.

At this point I would like to raise a word of caution to the reader. Although morphological changes indicative of tissue and cellular activity unfold in complex patterns over time, the temporal dimension is usually missing to the neuropathologist examining an autopsied brain or a single brain biopsy. In this regard, a salient limitation to the neuropathologist's assessment is that in the majority of cases, diagnosis is based on observations at a fixed time point. The postmortem examination of gross and microscopic findings therefore portrays an interrupted view of an otherwise unfolding biological process.

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1.2 Reactions at the Cellular Level

The life history of cells can be defined in terms of a series of trade-offs between growth, maintenance, reproduction, and the acquisition of resources. These exigencies can vary dramatically among different cell types, especially when faced with limited resources. A scarcity of resources propitiates trade-offs thus markedly skewing the balance favoring one cell resource over another. This is specially so for the human brain, an organ that receives 15 % of the cardiac output and accounts for 20 % of the total body oxygen consumption.

Neurons exemplify the history of trade-offs, i.e., by taking away division the cell is able to allocate more resources into other functions such as overall maintenance and long-term survival of the cell. Thus, the difference between the neuron and other cells in the body is not all that different from observations at the species level. A tree swallow is a small bird that reproduces often and has a short life span. Not coincidentally an albatross is a fairly large bird that reproduces less but has a longer life span. Life strategies at different levels of complexity (cell → species) are not all that different from one another; rather, they represent different ways of maximizing survival. This adaptive response of neurons, aimed at maximizing cellular longevity at the expense of reproduction, helps explain the absence or scarcity of neoplastic processes involving these cells (Casanova 2005, 2010).

In general, the overall level of cellular activity is reflected primarily in nuclear features, while functional differentiation is best observed in characteristics of the cell body (e.g., modified ependymal cells in the papillary fronds of the choroid plexus, whorling for meningeal cells). For cells in their normal state of activity (i.e., euplasia), nuclear features are relatively uniform and predictable when comparing cells from different sites. Chromatin that is well preserved has sharp borders that are crisply delineated from the nucleoplasm. In effect, the sharp chromatin borders look almost as if made by a cookie-cutter. The nucleolus, when present, is taken as an indication of protein synthesis and a marker of metabolic activity. Surrounding the nucleus are numerous stacks of rough endoplasmic reticulum and free ribosomes. Because of their affinity to basic dyes, the rough endoplasmic reticulum and free ribosomes are also known as chromatophilic substance or tigroid/Nissl bodies. The chromatophilic substance, similar as to what is observed in other secretory cells, is prominent in pyramidal cells but can dissolve and disappear under certain pathological conditions (e.g., chromatolysis).

In euplasia the nuclear membrane is uniformly thick, a finding in contrast to the striking irregularities seen in some types of tumors. Cells lacking cytoplasmic features of differentiation are called undifferentiated and regardless of either tissue or site of origin may be difficult to tell apart, e.g., stem cells (Fig. 1.1a). Under the light microscope undifferentiated cells tend to have a high nucleocytoplasmic ratio. The lack of cytoplasmic differentiation may be predictive of their high mitotic potential.

The degree of change from the basal euplastic state is often dependent on a number of variables, including pH/lactic acidosis, temperature, and chronicity of the underlying pathological process. Progressive levels of cellular activity are often

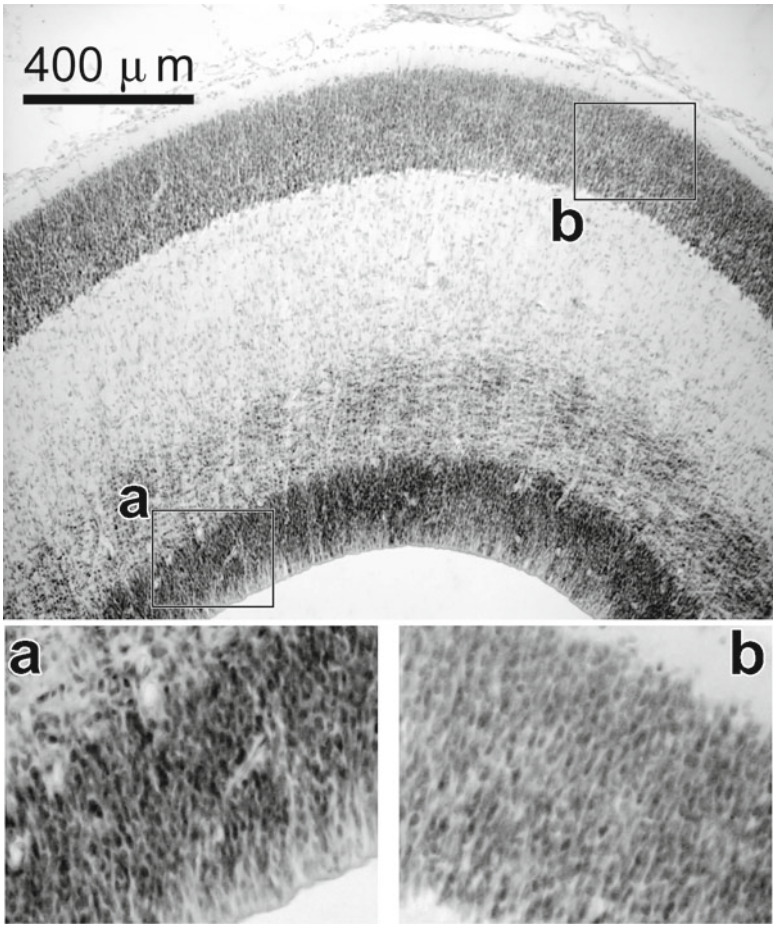

Fig. 1.1 The figure illustrates minicolumns during early human fetal development (12th week of gestation). Cell morphology is defined by the large nucleocytoplasmic ratio with little evidence in terms of somatic specialization. Despite their outward appearance, higher-resolution pictures show that there are no bare nuclei. The uniformity of the chromatin pattern, the tendency towards a round configuration, and the predictability of the cytoplasmic borders attest to their euplastic state. The prominence of Nissl staining within the cytoplasm reflects the amount of RNA in the same. The hyperchromasia of the nuclei and prominent Nissl staining of the cytoplasm define a high level of activity in an otherwise undifferentiated cell. These immature neurons or neuroblasts, precursors to pyramidal cells, acquire a rectilinear apposition to each other by migrating along a radial glial projection into the cortical plate. In this regard, positioning of neuroblasts antedates cellular and cortical specialization (e.g., synaptic contacts and laminar development). (a) Periventricular germinal cells and (b) minicolumns during corticogenesis

denominated as hypertrophy or hyperplasia. In clearly abnormal states of metabolic activity, some progressive changes are referred to as dysplasia or anaplasia. Both of these processes are characterized by disordered cell growth, loss of cytoarchitectural orientation, variations in size and shape of the nuclei and soma

(pleomorphism), and deeply staining nuclei (hyperchromasia). Both progressive and regressive changes may be observed concomitantly in some processes such as chronic irritation and inflammation. As expected, progressive changes typical of malignancy are best observed in the nucleus. In general, nuclear structures in malignancy (including nucleolus, chromatin, and nuclear membrane) are characterized by their angularity and irregularity.

Regressive changes imply a withering away of the cell. Early neurologists called it "abiotrophy" to signify a loss of a vital nutritive factor. In regressive states the optimal biochemical environment of the cell deviates from normal without itself becoming life-threatening. The insidious process provides for long-standing unfavorable living conditions that manifest itself as shrinkage of the cell soma and its projections as well as distortions in shape. Occasional mechanisms involved in regressive changes include the aging process, oxidative stress, and reduced protein synthesis (Kanazawa 2001).

Smaller cells have been described for both cortical and subcortical neurons in primary as well as syndromic autism (Bauman and Kemper 1985, 1994; Raymond et al. 1995; Casanova et al. 2006; Van Kooten et al. 2008; Wegiel et al. 2010). According to the literature smaller neurons provide for a variety of pathological phenotypes including developmental arrest, aposklesis, abiotrophy, and in some cases a type of non-apoptotic dark cell degeneration. More recently Casanova et al. (2011) have reported that in autism the smaller size of neurons correlates to the "even expansion" of minicolumns. This shift in minicolumnar/neuronal soma size biases brain connectivity towards an "intrahemispheric modus operandi" (Doty 2007, p. 282). In essence a decrease in pyramidal cell size favors shorter projections at the expense of longer-range, metabolically expensive projections.

It is important to distinguish regressive changes from those caused by tissue degradation, i.e., cells having lost features of cytoplasmic differentiation, occasional bare nuclei, and a blurred chromatin pattern. It is also imperative to recognize this change because the nuclear pattern may contain alarming features such as clumping of the chromatin and hyperchromasia which may be confused with a malignant cell having a high nucleocytoplasmic ratio. Recognizing these changes allows you to judge the suitability of tissue specimens for certain techniques such as autoradiography or in situ hybridization. The following are *apropos* remarks from senior autism researchers in regard to the quality of the postmortem tissue used in research:

> "…it was very disappointing to discover that the majority of the brain samples showed extensive degradation and that no meaningful conclusions could be drawn from the experiments. If we had not decided to perform the autoradiography and the hemalum staining after the Western blot experiments, we would have not been aware that we were working with degraded tissue samples. Several research groups received the same brain samples that we got and because they did not perform brain sections, they did not realize the problem with the tissue quality and went on to publish their findings." Catalina Betancur and Salah El Mestikawy, Université Pierre et Marie Curie, Paris France (Jane Pickett, ATP Report, 2010)

1.2.1 Lipofuscin

Lipofuscin is a membrane-bound pigment and a by-product of cellular waste. The pigment accumulates with aging in a nonuniform distribution. Preferential accumulation is seen in neurons of the dentate nucleus of the cerebellum and inferior olive, as well as in neurons of the anterior horn of the spinal cord. The old view of pigmentary atrophy suggested that lipofuscin accumulation could lead to cell death. Accumulating evidence in recent years seems to validate this point of view. Lipofuscin accumulation may impair multiple metabolic cascades including the ubiquitin/proteasome, leading to what has been called a "garbage catastrophe" (Terman 2001). Defective or imperfect clearance of indigestible material leads to their accumulation thus hindering catabolic and anabolic functions of the cell.

An early review of the neuropathology literature of autism by Darby (1976) found the most common finding in their series to be "cerebral lipidosis." The term was not meant, in a more modern sense, to define a sphingolipidosis (e.g., Tay-Sachs disease); rather, in the absence of an inheritance pattern, progressive spastic paralysis, or blindness, cerebral lipidosis was used to describe lipofuscin accumulation. A recent study quantitating the size of lipofuscin aggregates in autism revealed a significant increase in the number of pigmented cells (López-Hurtado and Prieto 2008). The authors believed that increased lipofuscin accumulation in autism is a marker of accelerated neuronal death or increased oxidative stress.

1.2.2 Gliosis

1.2.2.1 Astrocytes

There are multiple markers for astrocytes the most widely recognized being glial fibrillary acidic protein (GFAP). This is an intermediate filament protein involved in the structure and function of the cytoskeleton of both astrocytes and ependymal cells. Protoplasmic astrocytes of the cerebral cortex are usually GFAP negative. These astrocytes may proliferate under metabolic derangements such as hepatic or uremic coma (e.g., states of chronic hyperammonemia). By comparison, fibrous astrocytes are typical of the white matter and their cell soma will react, although incompletely, to GFAP. When reactive, the cell bodies of astrocytes undergo enlargement and lateralization of their nucleus. The appellation of reactive astrocytes as "gemistocytic" (from the Latin word gemmule for bud) denotes their occurrence in closely apposed pairs reflecting their proliferation. Astrocytes may interact with other tissue elements to restore glial margins around an injured site. The end result of this process is called glial scarring.

Astrocytosis, as shown by GFAP staining, denotes an acute process occurring at the time of tissue sampling. Certain conditions such as Wernicke's encephalopathy,

varicella zoster, and HIV infection decrease GFAP expression. Decrease expression has also been reported in certain psychiatric conditions such as schizophrenia, bipolar disorders, and depression (Johnston-Wilson et al. 2000).

A recent survey of brain specimens available from the Autism Tissue Program revealed that the majority of cases had suffered from hypoxic/ischemic deficits and/or reperfusion injury during their preagonal state (Casanova 2008). Reperfusion injury provides for disruption of the blood–brain barrier, inflammation, and oxidative damage. Free radicals destroy structural proteins and membrane lipids primarily within the white matter of the brain. Brain slice experiments indicate that oligodendrocytes are particularly susceptible to damage by free radicals (Husain and Juurlink 1995).

In autism, autopsy reports of GFAP-positive astrocytosis appear to preferentially affect the white matter of the brain. The GFAP staining portrays a reaction taking place close to the time of death rather than a signature of a neurodevelopmental condition. If the clinical history is not suggestive of an ongoing deteriorating process, such a finding always brings to mind the possible confound of a preagonal or agonal condition. It may well be that reports of astrocytosis in autism may provide a clue as to the way the patients died (e.g., ischemia–reperfusion injury) or comorbidity (e.g., seizures), rather than a mechanism as to the underlying condition. In this regard, a MAP2 immunocytochemical study found no difference in density of glial cells in the frontal cortex of two autistic individuals. There was increased glia of the subcortical white matter, but the same was primarily attributed to a long-standing history of seizures in one of the patients. The authors concluded that "in autism unaffected by additional pathology because of epilepsy, glial cells may be unaltered in the subcortical white matter. However, this observation needs to be explored further in a large sample" (Mukaetova-Ladinska et al. 2004, p. 621).

Much controversy has been created by reports of Purkinje cell loss in autism. Initially an early gestational lesion was presumed, in part, due to the lack of an astrocytic response. However, these observations were based on non-stereological assessments of Nissl stains. A modern immunocytochemical study has shown that the Nissl stains are biased techniques in that they do not identify the totality of cells they are meant to identify (Mukaetova-Ladinska et al. 2004). According to the latter authors, agonal events and postmortem handling can account for inadequate staining and lower Purkinje cell counts in previous studies (Whitney et al. 2008). Stains with higher specificity (GFAP) have conclusively shown marked reactive gliosis to Purkinje cell loss (Bailey et al. 1998; Whitney et al. 2008). The presence of GFAP-positive astrocytes as well as the cellular morphology denotes an acute reaction. Chronic injuries leading to fibrillary gliosis are best seen with Holzer or PTAH as a marked increase in astrocytic processes but little in terms of soma. In many of these cases, the reaction appears to be an attempt to heal the tissue by "filling in" a defect. That means that the gliosis accompanying Purkinje cell loss was acquired in the sense that it was actively taking place when the patients died or the tissue was retrieved. Given the fact that a significant portion of the patients suffer from seizures (in many unrecognized), receive medications for the same, or suffered from some

type of hypoxic/ischemic deficit, these confounds could offer the best explanation to Purkinje cell loss.

It is therefore not surprising that the modern view supports a model wherein Purkinje cells are lost after they are generated and migrated to their proper layer (Vargas et al. 2005). In this regard Purkinje cell loss in autism appears to be an acquired phenomenon not necessarily related to the core pathology of the condition. This observation may be of importance when judging the usefulness of animal models of autism that employ Purkinje cell loss as a pathological marker for the condition.

1.2.2.2 Microglia

Microglia make up 5–12 % of the total glial pool of cells. Although originally described by Pío del Río Hortega as mesenchymatous in origin, it is now accepted that they develop from hematogenous monocytes that invade the brain parenchyma during embryogenesis (Whitney et al. 2008). The resultant cells mature in the brain parenchyma as resting microglia. When activated, microglia acquire an ameboid-like cell body and are capable of releasing immunomodulatory compounds.

Morgan et al. (2012) used immunocytochemistry to study microglial activation in a single brain region (dorsolateral prefrontal cortex) of autistic patients. A limitation of the study was the use of different protocols for both tissue processing and sectioning within their series of autistic and controls individuals. Many of the reported parameters were related statistically to the processing technique. A more recent study by the same group reported a significant spatial relationship between microglia and neurons (Morgan et al. 2010). The authors were uncertain as to the significance of the results, that is, whether the spatial microglia-neuronal relationship was protective, pro-healing, or deleterious.

1.2.2.3 Gliosis, Plasticity, and Brain Development

Experiments in animals, mostly rodents and to a lesser extent primates, have expanded our knowledge of brain ontogeny. The idea that the immature central nervous system is more resistant to insults than mature ones probably first arose from the vacuum pump experiments of Robert Boyle at the turn of the seventeenth century. Later on Legallois, during the French Revolution quantified this observation by following the duration of gasping movements in decapitated animals as a function of time. However, it was not until the repeated efforts of Margaret Kennard (1936) in lesioned monkeys that the plasticity of the immature central nervous system became an attractive possibility. The notion was soon challenged that developmental brain lesions remained silent because they are either compensated behaviorally or the functional demands are redistributed. Researchers started to think in terms of an underlying structural organization in order to explain sparing of function in immature animals.

It is noteworthy that even though early lesions may spare the most overt motor and sensory functions, they may still express themselves with a characteristic, albeit harder to define symptomatology, for example; behavioral testing of humans with early brain injuries revealed unexpected cognitive retardation, mirror movements, and peculiar hyperesthesias. Furthermore, the magnitude of this abnormality seemed to be dependent both on the type and age of testing. Similar observations have been made in animals where one of the favorite models of central nervous system plasticity is that of unilateral collicular lesions and the resultant rerouting of the unaffected eye projection to the contralateral tectum. When presented with a visual stimulus to the affected eye, the animal exhibits misdirected movements. This abnormal behavior may be exaggerated or diminished by environmental manipulations.

Animals, including humans, show attenuated reactive gliosis during brain development. Depending on age of insult, lesions may show reduce scar formation and an improved regeneration of neuronal synapses. Mice exposed to unilateral hypoxia-ischemia suggest that attenuated gliosis in the developing brain does not affect the hemisphere or infarct volume; rather, it increases the number of surviving neurons (Kennard 1936).

In humans the developing brain can provide a gliotic response as early as 20 weeks of development (Järlestedt et al. 2010) and certainly throughout the third trimester of gestation (Roessmann and Gambetti 1986). Perinatal and postnatal brain injuries should result in a gliotic response. In schizophrenia the fact that the majority of studies suggest a lack of gliosis has been taken to support the so-called neurodevelopmental hypothesis for the condition. By contrast, studies of reactive gliosis in autism variously suggest (1) arguments against an early developmental lesion, (2) that autism is not a static encephalopathy, and/or (3) that two or more hits are required over the lifespan for disease development or progression. Another possibility, and probably the most adequate, as previously discussed (see above), is that gliosis in autism relates to comorbidity (e.g., seizures), medication usage, or pre-agonal/agonal events rather than to the core pathology of the condition.

1.3 Reactions at the Tissue Level

1.3.1 Necrosis

Necrosis is the death of brain tissue affecting to varying degrees all anatomical elements within a given area. The involved tissue most often liquefies. Contrary to apoptosis where the inciting agents are endogenous to the cells, provoking agents in necrosis are external to the involved tissue, e.g., trauma and infection. When the injury affects lysosomal enzymes and denatures structural proteins, the tissue is able to maintain, for a few days, a “coagulated” morphology with preservation of tissue architecture and cell outlines.

1.3.2 Edema

Edema constitutes the major response of the brain to most types of injuries. Three main classes of edema have been recognized: vasogenic, cellular (cytotoxic), and interstitial (hydrocephalic). Among these, vasogenic edema is probably the most important as it accompanies focal brain lesions such as primary cerebral tumors, metastases, and trauma. Research has revealed that vasogenic edema consists of an accumulation of plasma-like fluid in the extracellular space of the brain with preferential involvement of the white matter.

The venous vessels of the brain differ from those found at other locations of the body in regard to the thinness of their walls, absence of smooth muscle, and scarcity of elastic tissue. These characteristics combine with the brain's lack of a supportive tissue skeleton to make the capacitance vessels easily collapsible. This anatomical characteristic may underlie the pathophysiology of edema in certain conditions such as metastases and hematomas that may quickly become symptomatic due to their expanding mass growth effect. Contrariwise, infiltrative lesions, as seen in astrocytomas, would not be as likely to compress vessels because of the diffuse pressure they exert.

Besides the vasogenic type of edema previously discussed, other major types of edema are interstitial and cytotoxic (cellular). Interstitial edema occurs with obstructive hydrocephalus when there is transependymal flow of CSF into the white matter. Vasogenic edema differs from interstitial as the latter contains virtually no protein. Cytotoxic edema is characterized by swelling of cellular elements as a result of inadequate functioning of the sodium potassium ATP pump. Contrary to previously mentioned processes, there is preservation of the blood–brain barrier in cytotoxic edema.

In autism there is no evidence to suggest an excess accumulation of fluid within the intracellular or extracellular space of the brain. Similarly, there is no increased permeability of the vasculature and no evidence of leukocyte influx (transmigration) into the parenchyma, nor of phagocytosis. A couple of reports regarding isolated microglial nodules have been attributed to prior insults, e.g., encephalitis (Roberts 1991; Guérin et al. 1996). In occasional brain specimens reported swelling, unaccompanied by signs of herniation, appears to be an artifact of postmortem brain edema (Vargas et al. 2005) (see below).

1.3.3 Postmortem Edema

Both macro- and microencephaly have been reported as being found in higher proportions in patients with autism. Lainhart et al. (1997) reported that rates of head growth were abnormal in early and middle childhood in approximately one third of autistic individuals. Several studies have pursued Lainhart's findings using postmortem brain weights or neuroimaging. Unfortunately most reports don't specify whether reported brain weights were fresh or fixed. Formalin fixation provides for a 7–13 % gain in net brain weight in the first 5 days of fixation (Lainhart et al. 1997).

Higher gains have been reported with lower concentration of fixative (Quester and Schröder 1997) and when comparing children to adults.

Occasionally postmortem edema is the result of putrefaction. This process usually starts at the base of the brain. Injuries to the skull or the presence of septicemia enhances putrefaction. In putrefaction bacterial spread use proteins and carbohydrates from the blood as their culture media while taking advantage that protective mechanisms of the living body are absent. In autism, the study by Bailey et al. (1998) is significant, among other things, as being the only one to report the ratio of total brain to brainstem and cerebellar weight. Out of the six brains described by Bailey et al. (1998), three showed evidence of swelling without herniation (brain weights 1,600 g, 1,805 g, and 1,820 g) and may exemplify the artifact of postmortem brain edema. One brain in particular evidenced signs of putrefaction, being soft to the touch and containing numerous pockets of bacteria.

Studies on postmortem brain weights in autism have invariably failed to disclose relevant details that would have helped clarify the significance of their findings. More specifically, the narrative of gross findings fails to uncover any evidence of atrophy or swelling, and discussions almost never take into consideration the agonal and preagonal conditions of the patients. This limitation extends itself to other neuropathological studies in autism whose emphasis is not brain weight.

1.3.4 Mineralization

At the microscopic level, mineralization of the brain is usually recognized as basophilic globules tracking vessel walls. Although the intima of the vessels is usually preserved, on occasion it may proliferate to narrow the lumen. In severe cases mineralization encases the whole vessel wall with occasional deposits being found free within the neuropil. There is little, if any, accompanying cell loss, gliosis or tissue rarefaction. Histochemical analyses of these minerals disclose the presence of many elements (e.g., iron, calcium, zinc) within an organic matrix.

The role of iron within mineral deposits in the brain has drawn some interest within psychiatry. This is due to iron's modulatory role on the dopamine receptor and its role as a cofactor for tyrosine hydroxylase. Dopamine is not the only neurotransmitter by which iron may manifest symptoms. Iron colocalizes with gamma-aminobutyric acid (GABA), serotonin, and some neuropeptides, thus suggesting a role in the utilization of these neurotransmitters. Furthermore, many neuroleptics chelate iron and in chronic usage increase iron concentrations within the caudate nucleus. Iron may also play a role in neuronal degeneration by catalyzing the formation of oxygen radicals, induction of proteases, and increasing membrane lipid peroxidation.

Mineral deposits occur within a large spectrum of severity. Although most commonly affecting the globus pallidus, more severe cases involve the putamen and dentate nucleus. Small deposits tend to be asymptomatic. Extensive deposits tend to be associated with a gamut of neurological and psychiatric manifestations. Free radical reaction, iron deposition, and dopamine abnormalities may provide a

network of interrelated deficits linking brain mineralization to psychiatric manifestations (Bailey et al. 1998).

Tuberous sclerosis is a multisystemic disorder caused by the mutation of either of two genes, *TSC1* or *TSC2*, which regulate cell growth. It is inherited as an autosomal-dominant neurocutaneous disorder. Between 25 % and 61 % of affected individuals meet the diagnostic criteria for autism, with a higher percentage showing features of the broader phenotype of pervasive developmental disorders (Casanova and Araque 2003). Its neuropathology is characterized by subependymal nodules, giant cell astrocytomas, white matter heterotopias, and cortical tubers. Neuroimaging studies of tuberous sclerosis patients usually show calcified subependymal nodules and cortical tubers. The presence of calcified component in the tubers does not indicate a static lesion; lesions can and do change with time. Tubers in the temporal lobe appear more commonly in those patients with autistic manifestations than in those lacking the same (Harrison and Bolton 1997). This author believes that the correlation of tuberous sclerosis to autism may be due to subependymal nodules and disruption of the periventricular germinal epithelium during brain development.

1.4 Corticogenesis

The cortex arises from undifferentiated neuroepithelium that attaches itself to both the ventricular wall and the pia (Marín-Padilla 1995; Kothur et al. 2008). The terminal projections of these cells provide for a pia externa glia limiting layer and its basal lamina. Early arriving corticopetal fibers and primordial neurons, origins unknown, populate a marginal zone. These neurons will assume different morphologies according to their relative positions. Those that remain superficial acquire the horizontal morphology characteristic of Cajal-Retzius cells (Marín-Padilla 2010). Deeper lying primordial cells acquire pyramidal cell features, i.e., ascending apical dendrites and basal dendrites. Martinotti neurons will also first be observed in this deeper lamina but by birth are also seen in more superficial laminae. The composite arrangement of the periventricular neuroepithelium, first lamina, and subplate zone has shared features with the primitive cortex of both amphibians and reptiles.

Mitotic divisions of the ependymal epithelium will provide for migrating neuroblasts that accumulate in the cortical plate splitting the original preplate into a first lamina (I) towards the pial surface and a deeper subplate. Migrating neuroblasts use radial glial filaments as guides while being attracted by reelin from the Cajal–Retzius cells. Arriving at the cortical plate, neuroblasts loosen their connection to their radial glia guide and acquire an inside out type of positioning depending on previously migrated neuroblasts (Tissir et al. 2002). The apical dendrites of these neuroblasts will splay open as bouquets within the first lamina. The resultant cortical plate is considered a mammalian innovation.

When the germinal neuroepithelium finishes its asymmetric divisions, the resultant cortical plate is some 100 neurons thick with apical dendrites firmly anchored

within the first lamina. No more neuroblasts will be added to the cortex after 17–18 weeks of human gestation (Aboitiz 1999). The resultant scaffolding of future pyramidal cells retains a striking radial arrangement with cell soma-free space (neuropil) at its sides. With maturation and postnatal development, many of these cells will diverge from the central axis of the minicolumn. At least two studies have shown a temporal continuity between minicolumns of embryonic development and the pyramidal cell arrays observed in postnatal specimens (Krmpotić-Nemanić et al. 1984; Casanova et al. 2007). Specific aspects of the minicolumnopathy of autism are described in the next chapter (The Neuropathology of Autism) (Fig. 1.1).

In autism migrational abnormalities are suggested from accounts of cortical dysplasias, thickening of the cortex, variations in neuronal density, minicolumnar alterations, the presence of neurons in the molecular layer and within the white matter, irregular laminar patters, poor gray-white matter differentiation, and ectopic foci of cells (Marín-Padilla 2010). Neuronal migratory defects have been linked to abnormalities in brain growth and cortical organization, both of which are closely tied to gyrification.

1.5 Cytoarchitectonics

The fact that a significant amount of pathology escapes the level of gross brain inspection has helped define the topographical organization of the brain's cortex using microscopy. Early studies by Campbell (1905), Brodmann (1909), and Von Economo and Koskinas (1925) focused on the distribution and arrangement of individual cells (cytoarchitectonics), but other methods of parcellation have been variously proposed and pursued, e.g., chemo-, myelo-, and patho-architectonics. In cytoarchitectonics the complexity of cellular arrangements has served to challenge the concept of well-defined homogeneous brain parcellations. After extensive studies in different species, Bailey and Von Bonin (1951) indicated that attempts at dividing the vast majority of the neo- or isocortex was "unprofitable, if not impossible." Previous independent studies in the brains of two spider monkeys by Lashley and Clark (1946) bore little agreement to each other. In effect, the study showed that there was a large amount of variability among the areas, and some areas of the brain apparent in one specimen were missing from the other! In what must be considered a prescient observation, Lashley and Clark (1946) emphasized the need to corroborate cytoarchitectural fields with other methods.

Cytoarchitectural parcellations are not defined in terms of gyral location but by their particular cellular characteristics. Brodmann's map is particularly uninformative as it was based on a single specimen and therefore did not take into account marked interindividual variations. It is also uninformative in lacking descriptions of cytoarchitecture within the banks of the cortical sulci wherein a large percentage of the surface lies hidden. Researchers often misuse Brodmann's map as a way of labeling sites in functional imaging studies that relate areas of activation to a template image that exemplifies an invariant parcellation scheme.

A similar misuse of brain parcellation schemes are the attempts at absolute quantitation of cells and other anatomical elements within defined cortical areas. Besides the interindividual variability already discussed, many such attempts do not take into account the transition areas between different brain regions. In effect, cytoarchitectural areas are not neatly defined throughout their territory but rather suffuse in a subtle manner with neighboring sites. These transition areas may comprise a significant percentage of the cerebral cortex and have led researchers to suggest that the boundaries of brain areas defined by cytoarchitecture should have confidence bars superimposed on them (Lashley and Clark 1946). The lack of well-defined borders for the different cytoarchitectonically defined areas leads to a recognition bias for techniques such as stereology, which require unambiguous definition of the boundaries of reference space. A well-defined reference space allows the investigator to define both biological variation and sampling error. This information is necessary when optimizing sampling design for maximum efficiency (Casanova and Kleinman 1990).

1.6 Stereology

Quantitative aspects of neuronomorphometry usually demand the application of stereological principles. Stereology refers to the application of geometrical accepted truths or theorems to two-dimensional measurements. It allows an investigator to estimate in an assumption-free or unbiased manner descriptive parameters of a structure, e.g., number, length, surface, and volume. Typically, sectioning three-dimensional objects with a plane and sampling the resultant structure with specialized probes generates measurements of geometrical properties. These probes extract features from an image that produce outcomes of stereological interest. The probe recommended for use in estimating numbers in thick sections is the optical dissector probe. The estimates are calculated from counts obtained by systematic random sampling of a particular well-defined area. Using a strategy described by West (1993) can help optimize the number of sections used and the intra-sectional spacing of the optical dissectors. Cells are counted using a fractionator and following certain rules:

- Neuronal nuclei completely inside the counting box are counted. I usually count nuclei rather than nucleoli or cell bodies, since their edges are more distinct than edges of cell somas and there is only one nucleus per cell in contrast to nucleoli.
- Nuclei completely outside the box are not counted.
- Nuclei that intersect the exclusion lines or the upper guard zone (the forbidden planes) are not counted.
- Nuclei that intersect the inclusion lines or the lower guard zone are counted provided they do not also intersect any forbidden planes.

The use of immunocytochemistry to quantify cells counters the basis of stereological work which by definition is assumption-free and unbiased. It is well known

that immunocytochemical techniques are not necessarily quantitative which explains, in part, why in pathology laboratories examining human tissues controls are used. Incubation conditions in immunocytochemistry seldom allow the intensity of staining to vary directly with the amount of antigen, nor do they prevent excess diffusion of antibody. In immunocytochemistry the lack of covalent bonds between antigen and antibody propitiates an equilibrium reaction that may be modified by factors affecting the overall condition of the tissue, e.g., postmortem interval, type of fixative, length of fixation, and tissue processing (West 1993). Even if these obstacles could be controlled for, immunocytochemical reactions should be run under standard conditions that maximize signal-to-noise ratio [e.g., optimal incubation times, using a saturating solution of the chemical marker (e.g., diaminobenzidine), and preventing excess diffusion of the antibody]. Lack of immunocytochemical staining, under optimized conditions, does not exclude the presence of antigen. The failure of immunocytochemical staining to reveal quantitative features of the object of biological interest is known in stereology as a recognition bias. This bias can't be quantitated, corrected, or removed when performing stereology.

Besides cell counts, volumetric studies have been offered as an index of pathology. These studies have limited usefulness. The classic study of Konigsmark on the ventral cochlear nuclei failed to find a correlation between overall volume and number of neurons (Casanova and Kleinman 1990). Similar studies have been performed in autism claiming pathological significance to the so-called hypo- or hyperplasia of the vermis and/or cerebellar hemispheres without looking at Purkinje cell numbers. Asseverations from such studies have limited value. It is not surprising that hypoplasia of the vermis has been reported in a number of neurologic disorders and is not specific to autism.

1.7 Neuropathology and Animal Models

The relevance of animal experiment to human pathology should be restricted due to a number of considerations. For instance, the neuronal networks of animals and humans are different. Thus, brain lesions in rats or rabbits do not show a contralateral hemiparesis, whereas human brain lesions do show a corresponding paralysis. Furthermore, experiments do not place demands on animals comparable to those of life experiences on human subjects nor do they take into account the highly structured stimulus–response patterns of lower species in comparison to the greater flexibility exhibited by species "higher" in the evolutionary scale. Still, according to the aforementioned observations, it seems safe to assume that, if autism is the result of a neurodevelopmental lesion, its manifestations will vary according to the age when the affected individual is tested and that the degree of overt manifestation will vary depending upon the life experiences of that individual.

Is the brain of a mouse similar to that of a human? The answer is a definite "no"! There is a thousandfold difference in volume. According to the radial hypothesis, corticalization is the product of supernumerary minicolumns. In order for the added

minicolumns to be operational, they need to be interconnected. This increased need for corticocortical projections by supernumerary minicolumns causes larger brains to scale their white matter at 4/3 power of the volume of gray matter. Many of these fibers take the form of short corticocortical projections. Otherwise, corticalization has resulted in a relative decrease of longer commissural connections, such as the corpus callosum. This means that the blueprint of connectivity for small brains species differs markedly from that of species with larger brains.

The supernumerary addition of minicolumns in corticalization provides for an increase in the surface area of the gray matter and complexity in its pattern of folding. These changes occur with a relatively larger increase in white matter and a bias favoring short connections at the expense of longer ones. This correlation is exemplified by studies showing a direct correlation between the gyral window (i.e., aperture at the base of the gyri that allows projections in and out of cortex) and size of the corpus callosum. A fast conducting large-diameter myelinated fiber may occupy 10,000 the volume of the finest unmyelinated fiber of the same length. Both space and metabolic considerations make long-range connections expensive to maintain as evolution has placed an onus on larger brains.

Encephalization has provided for differences among species in cortical surface area, folding (gyrification), parcellation, and connectivity. Differences may vary according to their location, e.g., while the visual cortex doubles in size when comparing macaques and humans, the volumes of the frontal and parietal lobes differ by 10- to 40-fold. The small lissencephalic brain of mice has about a dozen or so well-recognized cytoarchitectural areas, while in the human primate the number of brain regions is only limited by the imagination of researchers. Primates, in particular, are unusual among mammals in having a region within the prefrontal cortex with a well-developed internal granular layer (granular frontal cortex). Because of its rich connectivity and crucial role in executive functions, this region (i.e., dorsolateral prefrontal cortex) has been implicated in a large number of psychiatric conditions. Animal models of psychiatric conditions occasionally call attention to changes in this cortical region unknowing that this area is absent in the proposed model. Similarly, rodent animal models also lack the cellular representation for anatomical elements incriminated in the pathophysiology of autism, e.g., double bouquet cells and von Economo neurons.

1.7.1 Turing Test for Animal Models

Alan Turing was a Cambridge don who is best remembered as a famous code breaker during World War II. He was also a proponent that computers, given enough code, would one day be able to display logical thought. In order to test this assertion, he proposed a test where a human judge, blind to the conditions of the experiment, would engage via a keyboard and screen a human and a computer in natural language conversation. If the judge could not reliably tell the human and computer apart, the machine would be deemed to be intelligent.

We could probably envision a similar test to judge the validity of animal models. The test would be a top-down endeavor wherein an examiner would blindly examine a number of different animal models and try to diagnose the same as being a model for a human condition, e.g., autism, ADHD, obsessive–compulsive disorder, etc. Even in knockout models of known genetic conditions, passing such a test would be difficult. In this regard animal models copy a limited range of behaviors but seldom a human condition.

1.8 Summary

Despite the importance of neuropathological research, the scarcity of articles in the autism literature borders on lack of interest. It may be that neuropathological techniques are specialized, and it may be easier to design studies in other areas of research (e.g., neuroimaging) or to pursue those that promise monetary rewards in funding by different agencies (e.g., genetics). It has been said that when psychiatry stood away from neuropathology, it allowed for other perspectives to control the world view regarding the etiology of autism. It may not come as a surprise that lulls in interest regarding neuropathology have been dominated by psychoanalytic tendencies within psychiatry.

Unfortunately the lack of adequately trained individuals has given rise to a permissive attitude thus allowing untrained individuals to try their hand in postmortem studies. It is not that we have few findings regarding the neuropathology of autism; rather, we have too many. Some of the criticisms voiced in this chapter are meant to convey the fact that the core neuropathology of autism is hidden behind a myriad of accessory findings now reported within the medical literature. Some published results may be explained by comorbidity, drug usage, and preagonal/agonal condition of the patients. In this regard, neuropathology echoes the unsettling remark by Dostoyevsky that if there is no God, then everything is allowed. If there is no neuropathology, then we have to consider favorably every result and conclusion. This author has previously stated that this approach preserves falsehood by building tale upon tale.

When interpreting postmortem findings, please bear the following in mind:

1. Are the patients and controls adequately matched? Always consider the history of seizures, medications, and preagonal/agonal conditions of the patients. A majority of autism donors have suffered from hypoxia or ischemia–reperfusion injury. If the series are not matched for all of these conditions, can the results reflect the effects of these confounds?
2. Always be wary of postmortem studies that use inappropriate techniques or experimental designs:

   (a). Golgi studies in postmortem tissue are notorious for the amount of artifacts and the capriciousness of the impregnation. Attempts at quantitation using

postmortem tissue and the Golgi technique only expose the naivety of the researcher.

(b). Always examine the quality of the tissue before interpreting results of autoradiography or in situ hybridization. Several measures of tissue quality should be provided, knowing that bad results in a single one of them trumps negative results in the others.

(c). Know the limitation of the techniques. It is difficult to justify the use of stereology for quantitating aspects of postmortem immunocytochemistry. Similarly, using stereological counts on parcellated regions of the cerebral cortex is an incredibly difficult endeavor. Unless the authors have overcome the lack of cookie-cutter borders for parcellated regions, cytoarchitectonics of the cerebral cortex engenders a recognition bias not amenable to stereological studies.

Despite many limitations, neuropathology offers the best opportunity to unravel the etiology of autism. It is an exciting field where trained individuals can read the mechanics of life events, from neurodevelopment to the senium, in a single microscopic slide. The availability of multiple stains and techniques offers varied perspectives to suit individual neuropathologists. Each slide builds a story; the art of neuropathology is in putting the story line together.

The Pathology of Autism, Is It Strictly a "Genetic Disorder"?

By Paul H. Patterson, Ph.D.

Talks and papers by geneticists working on autism frequently begin with a variant of the phrase "autism is a genetic disease." My understanding of the term "genetic disease" is that it applies to disorders in which a mutation invariably leads to the disease, such as in Huntington's. Some disorders, such as fragile X, are monogenic and include features of autism, but the overall phenotype is clearly distinct from idiopathic or sporadic autism. There are also some very rare mutations and copy number variants that cause autism, but these account for only a small fraction of the disorder. This situation has been termed "The mystery of the missing genes" or "Where did all the heritability go?"

The assertion that autism is a genetic disease is often justified by a statement that the concordance between monozygotic twins is 90 % when one twin is diagnosed with the broader phenotype of autism spectrum disorder (ASD), while the concordance between dizygotic twins is near zero. These numbers are based primarily on older twin studies. However, a new, very large twin study used contemporary standards of diagnosis (ADOS and ADI-R) and came up with rather different numbers. For strict autism, probandwise concordance for monozygotic male twins was 58 % and 21 % for dizygotic pairs. For female twins, the concordance was 60 % and 27 % for dizygotic pairs. For ASD, the probandwise concordance for male twins was 77 % and 31 % for dizygotic pairs. For female twins, the concordance was 50 % and 36 % for dizygotic pairs (Konigsmark and Murphy 1972). Not only are the figures for monozygotic pairs considerably lower than older studies, but the concordance for dizygotic pairs is much higher than for siblings (sibling concordance values

Table 1.1 Comparison of the effect sizes for schizophrenia for various maternal infections and candidate genes (data courtesy of Alan Brown)

Maternal infection	Odds ratio	Genes	Odds ratio
Influenza—first half of pregnancy	3.0	*NRG1*	1.1–1.2
Toxoplasmosis	2.6	*DISC1*	1.1–1.2
Genital/reproductive—periconception	5.3	*DTNBP1*	0.9–2.7
Respiratory—second trimester	2.1	*COMT*	1.1
		MHC class I sequences—SNPs	1.1–1.3

have ranged from 5 % to 18 %). These values agree with findings from another very large twin study (Hallmayer et al. 2011), which reported a dizygotic concordance of 31 % for ASD. The significant discrepancy between ASD risk for non-twin siblings versus dizygotic twins leads to the hypothesis that the intrauterine environment plays a key role in the risk for autism. Indeed, recent evidence reveals an association between ASD in the offspring and the presence of inflammatory markers in maternal serum or amniotic fluid (Rosenberg et al. 2009; Brown et al. 2013). Moreover, maternal infection during the first trimester increases the risk for ASD in the offspring (Goines et al. 2011).

Another mental disorder that is often cited as being highly heritable is schizophrenia, and here also there is the mystery of the missing genes. Moreover, there are well-documented associations between maternal infection and schizophrenia in the offspring, as well as associations between anti-flu antibodies or cytokines in maternal serum and increased risk for schizophrenia in the offspring (Atladóttir et al. 2010). In fact, extensive epidemiologic work has shown that the effect sizes of various maternal infections are considerably larger than the most studied candidate genes for schizophrenia (Table 1.1). In fact, summing the risks for these various infections suggests that >30 % of schizophrenia cases could be prevented if maternal infections were eliminated (attributable proportion calculation) (Brown and Derkits 2009). In further support of the influence of the maternal–fetal environment, indirect evidence indicates that the concordance rate for SZ appears to be much higher for monochorionic twins, which share a placenta, than for dichorionic twins, which do not share a placenta (Brown and Derkits 2009).

It is important to take these epidemiological findings into account in order to more accurately balance the relative importance of genes and environment in autism. For instance, overstating the importance of genetics has implications for understanding the developmental origins of this disorder. A great deal has been made of findings that many of the genes suggested to be involved in autism code for proteins that function at synapses and that the excitatory–inhibitory balance is important. How could synapses not be relevant for mental illness? Emphasizing genetics relative to environmental influences has also influenced research funding. Enormous expenditures have been (and are still being) made in the search for the ever-elusive candidate genes, while far less is being spent on the epidemiology of a variety of environmental risk factors and the pathophysiologies underlying them using animal models.

Is it not simply a truism that genes and environment (both surrounding the fetus and encountered later by the offspring) must interact to yield the autism phenotype(s)? This should be reflected in correcting the balance of funding as well as the mislabeling of autism as a "genetic disorder." Heritability estimates based on out-of-date twin studies should also be revised.

The Importance of Postmortem Research in Autism

By Eric London, M.D.

In the past 20 or so years that I have been involved with autism, as a parent, a researcher, a clinician, and a funder, I have seen the field take off from a backwater of the medical and research field to a place of prominence. Parents often ask "is there anything new and exciting coming out of the research?" By that, I believe they mean is there anything emerging from the research that is useful and could improve the lives of their children. During these 20 years, the progress made in treating autism has been rather meager. In 1989 the year I became interested in autism, the treatment of choice was applied behavior analysis (ABA) as outlined by Lovass along with some evidence that some medications were beneficial, most specifically haloperidol. In 2012 the treatment of choice remains ABA with some modifications and improvements, along with the medications risperidone or aripiprazole, both of which are similar to haloperidol. Many treatments and strategies have been attempted; however, research evidence for their benefit is lacking. Many widely used treatments have not even been researched, while others which seem to be promising show no benefit when careful research methods are used in an attempt to document the benefit.

Autism is not alone in being stuck in terms of progress, and the issue appears to pervade psychiatric disorders. Akil et al. (2010) directly address this question noting that there have been no major breakthroughs in schizophrenia in the last 50 years and no breakthroughs in the treatment of depression in the past 20 years. It has been noted that the pace of psychotropic drugs discoveries in the 1950s and 1960s was dizzying, whereas progress came to a halt over the past 40 years. Despite this lack of success there has been great strides made by the more basic scientists. Our progress in genetics and molecular biology has been dizzying.

I would like to suggest that one of the major factors holding back progress is a lack of high-quality "translational" science, and by that I mean the ability to relate the basic science findings directly to the disease state. There are three major strategies for translational research, one is genetics, the second is animal models, and the third is the research on human tissue. To be sure, in some diseases such as Rett's syndrome, great strides have been made mostly due to the discovery of the centrality of the *MECP2* gene. Genetics however, despite dominating the funding of the NIH over the past few decades, has contributed little to the understanding of the more complex brain disorders such as autism. While the search goes on, autism's apparent phenotypic and etiologic heterogeneity is causing many to become dubious about the prospects of success. Animal models also present some serious and perhaps insurmountable difficulties. Many question the suitability of using the

lower primates such as mice or rats to model diseases focused on functions which are only present in higher species such as language development. Research on the great apes and monkeys are difficult and expensive thereby loosing much of the advantage that rodent studies provide. Human tissue on the other hand offers a direct look at the disease processes themselves. For reasons not clear at all, there has been a pernicious neglect of support and funding this type of research.

The only NIH spending on human tissue banks for developmental disabilities is the Maryland Brain Bank, which is funded by the NICHD. They have a contract for funding at the rate of 7.15 million dollars for 5 years or about 1.4 million dollars per year. According to the NIH Report file which categorizes spending by categories, the NIH is estimated to spend in 2012 approximately 1.18 billion dollars on categories related to developmental disorders. The developmental disabilities list includes autism, CP, Battens's, conditions affecting the unborn, Down's, muscular dystrophy, epilepsy, fetal alcohol syndrome, fragile X, infant mortality, and perinatal problems related to low birth weight, intellectual and developmental disabilities, Rett's, and SIDS. Therefore, the investment in tissue banking for these diseases is 0.07 % of the portfolio. Similarly the amount spent by the NIH on tissue banking for DD is 0.02 % of the budget spent on genetics research.

It is exceedingly difficult for an individual scientist to provide his own tissue for research. While animal modelers can buy mice commercially, from agencies set up to provide these animals, no such system exists for human tissue. In addition, the tissue is worth little without the clinical data associated with that tissue. A careful system of brain collection, characterization, treatment, neuropathology, and distribution must be in place for the tissue to be utilized commensurate with its potential value. I am sorry to say that such a system is not in place to any significant extent.

Society has made several decisions over the years that have hurt research on human tissue. The first of which is the dramatic decrease in the numbers of autopsies done. From a clinical perspective this has hurt the ability of doctors to learn about pathology, especially in the cases where information is not forthcoming from premorbid testing. This is especially true for organs such as the brain or the heart which are not amenable for biopsy.

The primary factor in the decline in hospital autopsy rates is due to clinicians not wanting them. However, the discrepancy rate between the cause of death offered by clinicians and that shown on autopsy is 10–30 %. Increasing amounts of litigation however place a damper on the enthusiasm for the autopsy, at the cost of physicians not learning from their mistakes. The lack of enthusiasm for autopsies has been compounded by the adverse media attention to the retention of organs for illegal economic gain. All sorts of legal protections have been instituted, most of which has little to do with brain collection not used for transplant. Nevertheless, most governmental jurisdictions would rather make blanket laws and pay little attention to the research sacrifice being made.

While some point to resistance of the donor families, I have not seen this as a major issue. My experience with the families of autism cases who died is that the families feel that the tissue is a necessary step to reach an understanding of the disease that they had struggled with.

Although postmortem techniques have been used for hundreds of years, a new generation of systematic quantitative tools is still in its infancy. Tissue work remains the only way to study microscopic anatomy and cellular characteristics such as cell number and quantifying the length of dendritic processes. Microanatomic detail and brain region organization can give important information about developmental anomalies. Scanning technology allows the visualization of only grosser structures. A voxel resolution is about 1 mm, compared to a pyramidal neuron which is about 10 μm–50 μm. Molecular studies can identify, measure, and localize proteins, neurotransmitter receptors, or genes expressed in cells in a given region of the brain. Gene products and epigenetic studies cannot be done in peripheral tissue or animal models.

The very few postmortem tissue studies of autism published are very frequently cited papers. Animal models are based on postmortem reports of only a few cases, often using tissue of dubious quality or even equivocal diagnosis. Why funding is available for the models of autism and not for similar work on the autism cases themselves speaks to skewed priorities of our funding systems. If there is not a remedy to this problem in the near future, it is likely that we will have a generation of researchers who have no knowledge of how to handle or use human tissue, I believe, delaying progress in understanding these diseases by years or decades.

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Biography

Manuel F. Casanova is a board-certified neurologist trained in clinical electroencephalography and evoked response potentials. His research focus is autism spectrum disorders. Dr. Casanova is an endowed chair professor and is the associate chair for research in the Department of Psychiatry and Behavioral Sciences at the University of Louisville. He has over 20 years of experience in the neurosciences. During the last 5 years, he has published 43 refereed articles, edited 3 books, wrote 4 letters to the editor, and has completed 74 congressional presentations worldwide. He is one of the founders of the Autism Center at the University of Louisville. He was principal investigator on several federal grants, and now he is a PI on an NIH Eureka grant aimed at the application of TMS in autism.

Paul H. Patterson is the Anne and Benjamin Biaggini Professor of Biological Sciences at the California Institute of Technology. He attended Grinnell College in Iowa and obtained a Ph.D. at Johns Hopkins University. He began his work in developmental neurobiology while as a faculty at Harvard Medical School. At Caltech, his group developed a mouse model of mental illness based on the risk factor of maternal infection. His laboratory also has current projects on multiple sclerosis, Rett syndrome, and Huntington's disease. Patterson is currently serving on the scientific advisory boards of the International Rett Syndrome, the John Douglas French Alzheimer's, the Autism Speaks, and the Hereditary Disease foundations.

Dr. Eric London is the Director of the Autism Treatment Research Lab at the NY State Institute for Basic Research in Developmental Disorders. In 1994 he was the cofounder of the National Alliance for Autism Research which was the first major organization to fund basic and clinical research on autism. Early on, one of the major programs of this organization was the Autism Tissue Program which was organized to primarily to support the liaison between families and tissue researchers through the recruitment and distribution of tissue. The Autism Tissue Program continues under the auspices of Autism Speaks. As a clinical psychiatrist he has treated thousands of patients with autism and related disorders. Currently his research interests are looking for early predictors of autism, new models for service delivery, and re-conceptualizing the diagnosis of autism to provide a more clear and accurate phenotypic schema for clinical and research purposes. He is the father of a 25-year-old son with autism.

Chapter 2
The Neuropathology of Autism

Manuel F. Casanova and Jane Pickett

2.1 Introduction

Autism is a neurodevelopmental condition presently defined by operational criteria that, by themselves, lack in terms of construct validity. These operational criteria necessitate the screening of a variety of behavioral domains, e.g., communication, motor, and social skills. The diversity of behavioral domains that appear affected in autism makes this a Pervasive Developmental Disorder. By way of contrast, specific developmental disorders refer to explicit learning disabilities and other disorders affecting coordination. Specific developmental disorders may be subsumed under pervasive ones, and it is not unusual to have learning disorders, for example, in autism (Williams and Casanova 2012).

The term autism spectrum disorders (ASD) is used to describe three conditions with shared core symptoms: autism, Asperger, and Pervasive Developmental Disorders-Not Otherwise Specified (PDD-NOS). The existence of a PDD-NOS diagnosis prevents falsifiability for the diagnostic criteria of autism by sweeping atypical cases into this isolated category. The lack of construct validity to our classification schemes predisposes most studies to reaffirm the original observations upon which the criteria are based (Kanner 1943).

Autism is often seen in the presence of other medical/psychiatric conditions (for review, see Casanova 2007). Mental retardation and seizures are common comorbidities. Chromosomal abnormalities are frequent with certain genotypes having

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a significant higher incidence of manifesting autistic symptomatology. These genotypes include tuberous sclerosis, fragile X, Down, velocardiofacial, and Möbius syndrome. Much research has been done in regard to the possible link of tuberous sclerosis and autism. The coincidence in symptomatology should be expected as both tuberous sclerosis and autism share in widely distributed migrational abnormalities.

The comorbidity with chromosomal disorders attests to the neurodevelopmental nature of autism. The large number of comorbid conditions and susceptibility genes is a reflection of the clinical heterogeneity of autism and the potential for multiple underlying etiologies. Current medical understanding regards autism as a multifactorial or complex condition. Studies suggest that besides multiple susceptibility and protective genes, environmental influences play a significant role in its etiopathogenesis. As in many other multifactorial conditions, the risk of developing autism among first-degree relatives is higher than within the normal population.

The author has already suggested that, similar to other multifactorial conditions, autism offers a threshold phenomenon wherein three main factors impinge on each other to various degrees before the phenotype is able to supervene. The factors for the so-called triple-hit hypothesis are (1) a critical period of brain development, (2) an underlying vulnerability (e.g., genes), and (3) an exogenous stressor or stressors (Casanova 2007). The following sections will broach the subjects of gross and microscopic pathology before discussing the role of cortical modularity in autism.

2.2 Gross Neuropathology

Although a large number of structural abnormalities have been reported in autism, only a few have been reproduced by independent investigators. Among the more salient manifestation are increased brain size, complexity of gyrification, and diminished size of the corpus callosum. Increased brain size occurs without concomitant signs of edema (Casanova 2007). The volumetric increase does not appear to be a postmortem artifact as it has been reported in vivo with neuroimaging techniques and, in addition, increased brain size has been reported in first-degree relatives of affected individuals (Woodhouse et al. 1996; Fidler et al. 2000). When present, brain enlargement appears to be generalized, with conflicting data regarding the putative role of the cerebellum within the volumetric increase (Courchesne et al. 2001; Sparks et al. 2002). The findings of either hyper- or hypoplasia of the vermian lobules in different subgroups of autistic individuals remain controversial (Courchesne et al. 1988, 1994). The cerebellar findings have not been reproduced by several groups and do not appear to be specific to autism as they have now been reported in fragile X syndrome (Schaefer et al. 1996). Piven et al. (1992), when correcting for IQ among their comparison groups, reported no difference in vermian lobule size.

In a postmortem study of 19 cases by Kemper and Bauman, eight of eleven subjects under 12 years of age had increased brain size as compared to controls

(Kemper 1988). By comparison, six of eight autistic individuals over 18 years of age showed reduced brain size. Cross-sectional MRI studies now suggest that brain volume in autistic individuals increases during early childhood with the rate decelerating by late childhood and adolescence when brain volumes of autistic and controls become similar (Courchesne et al. 2001; Hardan et al. 2001; Aylward et al. 2002).

The presence of macroencephaly in autism persists after controlling for height, gender, and other medical disorders including seizures (Piven et al. 1995; Fombonne et al. 1999). The relationship between macroencephaly and IQ is unclear (Piven et al. 1995; Lainhart et al. 1997; Stevenson et al. 1997). In a recent review of the neuroimaging literature, Goldberg et al. (1999) found few replicated findings and criticized published studies for not controlling for confounding variables. Goldberg et al. concluded that the only independently corroborated findings were macroencephaly and decreased size of the corpus callosum, primarily the splenium.

Gross inspection of the brain has revealed few abnormalities. The gyral pattern of the brain of autistic individuals appears normal. There is a report of increased gyrification in the frontal lobes of autistic subjects using a single anatomical level for comparison (Hardan et al. 2004). Similar studies using another anthropometric measurement, the gyral window, have revealed smaller dimensions to this compartment (Casanova et al. 2009b). The gyral window is the space that constrains the passage of cortical afferent and efferents. A smaller gyral window presumably biases the size of its fibers favoring short connections (e.g., arcuate) at the expense of longer ones (e.g., commissural).

Some morphometric studies suggest that the white matter is disproportionally enlarged in regard to the gray matter (Herbert et al. 2003). Independent studies have now corroborated that the outer radiate compartment, containing short projecting axons (e.g., arcuate fibers), accounts to a significant extent for the increased white matter (Herbert et al. 2004). The inner or deeper white matter consisting of longer projections, e.g., commissural fibers, is diminished as shown by the reduced size of the corpus callosum. Casanova has suggested that the increase in outer radiate white matter is the result of supernumerary minicolumns in need of short-range connections (Casanova 2004).

2.3 Microscopic Pathology

Bauman and Kemper's classic study surveyed whole-brain celloidin-embedded sections (Kemper 1988). Each section was Nissl stained and cut at 35 μm following a protocol originally designed for the Yakovlev Collection. Sections were examined with a stereomicroscope that allowed side-by-side comparisons of autistic and control slides. Mounting and staining the free-floating sections provided for a good number of artifacts primarily affecting the cortex. Most of the detailed examination was therefore spent studying subcortical structures. Of the cortical sections examined, Bauman and Kemper reported no abnormalities in neuronal morphometry, lamination, and cellular density. Reported findings were primarily within the limbic

system (e.g., hippocampus, amygdala, mammillary bodies, septal nuclei) and cerebellum (Bauman and Kemper 1985, 1994). Neuropathology found in these areas included increased cell-packing density and reduced neuronal size. Within the cerebellum, both Purkinje and granule cells were found to be decreased in numbers throughout the hemispheres without any evidence of reactive gliosis. Furthermore, the olivary nuclei failed to show atrophy as expected with Purkinje cell loss. Four of the six autistic patients suffered from seizures, but the reported abnormalities were said to be similar regardless of the presence or absence of this comorbidity (Bauman and Kemper 1994). Bauman and Kemper concluded that the described features were characteristic of a curtailment of normal development.

Bauman and Kemper also examined Golgi-impregnated hippocampal sections of two autistic subjects and an equal number of controls (Raymond et al. 1995). Only one autistic subject was of good-enough quality to allow for analysis. The patient showed smaller somas in the CA4 hippocampal subfield. This report as well as the previous one with Nissl were based on subjective appraisals that relied on biased (non-stereological) assumptions. The small neurons reported by Bauman and Kemper in various regions of the limbic system may represent, as they stated, a "developmental phenotype." Other possibilities include *aposklesis* (cell withering usually associated with neurodegenerations) or a type of non-apoptotic dark degenerating cell. More recent studies using stereological techniques and well-defined anatomical criteria to define the subnuclei of the amygdala failed to reproduce the cell-packing results originally claimed by Bauman and Kemper (Schumann and Amaral 2005, 2006).

The nature of Purkinje cell loss in autism remains disputed. Although Bauman and Kemper insisted that the cell loss was part of a neurodevelopmental condition, the cerebellar foliar pattern remained normal and without additional evidence of disorganization of the remaining cellular elements (Harding and Copp 1997). This is the case even for the patches within the Purkinje cell layer where cell loss has been noted. More recent studies using immunocytochemistry (Bauman and Kemper used a Nissl stain) have shown marked glial proliferation as a reaction to Purkinje cell loss. Both the nature of the gliotic response and the use of GFAP staining denote a reactive process still undergoing at the time of death. The Purkinje cell loss may therefore be an acquired (postnatal) phenomenon explainable by seizures or the use of medications that exhibit neurotropism for the cerebellum, such as phenytoin (Dilantin) (Bailey et al. 1998; Pardo et al. 2005; Vargas et al. 2005).

Bailey et al. (1998) investigated the brains of six autistic cases (all mentally handicapped and three with epilepsy). Three of the brains were swollen, probably as a result of postmortem edema, one of which showed evidence of putrefaction. One case showed increased cell packing in all cornu ammonis subfields. Four cases showed areas of cortical abnormalities primarily involving the frontal lobes. This was the first report within the existing literature to incriminate a role for the cortex in the neuropathology of autism. The abnormalities reported by Bailey et al. (1998) included irregular laminar patterns, thickened cortex, increased number of neurons within the white matter, and heterotopias. The overall pattern was suggestive of cortical dysgenesis.

Similar to Bailey's report (see above) scattered postmortem and radiological data points to the presence of heterotopias in autism. Few magnetic resonance imaging (MRI) reports have indicated the presence of unidentified bright objects (Nowell et al. 1990; Bailey et al. 1998). Postmortem studies indicate their presence within the white matter and germinal zone (Bailey et al. 1998). All brain regions appear to be affected. Their presence, in terms of location, is highly variable among cases (Wegiel et al. 2010). The findings are suggestive of so-called epigenetic heterotopias as opposed to a genetically dictated condition. Previous authors have suggested that the presence of heterotopias in autism may help explain the link to seizures and tuberous sclerosis.

Several reports have suggested the presence of neuroinflammation in autism (Vargas et al. 2005). These reports are based primarily on the presence of reactive astrocytes and microglia. The classical inflammatory response involves a vascular component leading to the accumulation of hematopoietic cells and fluid within the extravascular space; vessels are engorged with margination of cells and the blood–brain barrier disrupted (Casanova 2007). As of present, there is no evidence that a classical inflammatory response is occurring in the brains of autistic individuals. Cerebrospinal fluid samples from live patients show normal results, including cellular components, protein electrophoresis, and levels of quinolinic acid and neopterin (Zimmerman et al. 2005). The reported findings do not support a role of tissue repair or recovery in the pathogenesis of autism.

The glial reaction observed in the brains of some autistic individuals may reflect, in part, their agonal conditions. A recent survey of available brains within the Autism Tissue Program (ATP) showed that the majority of patients died by drowning or incurred in other hypoxic conditions, e.g., seizures, sepsis, and anoxic encephalopathy (Casanova 2007). Reoxygenation of damaged tissue procreates a free radical cascade focusing on the rupture of double bonds as found primarily in membranes within the neatly arranged stacks of axonal bundles within the white matter. The end result is a gliotic response preferentially targeting the white matter. Agonal and preagonal conditions involving hypoxia and ischemia-reperfusion injury may therefore help explain some of the cellular response and the production of cytokines.

Hutsler et al. (2007) evaluated cortical thickness and lamination as proxy measurements of organization in eight ASD patients and a similar number of age-/sex-matched controls. There were no significant findings; i.e., average cortical thickness for any examined lobe was never greater than 3 % those of controls, and there was evidence of cell clustering in lamina I and subplate with little evidence of a defect in the lamination of the cerebral cortex. The same patients were later on used to study the gray-white matter boundary (Avino and Hutsler 2010). The results indicated an indistinct boundary in autistic patients believed to represent the presence of supernumerary neurons as a result of a migrational abnormality or failed apoptosis.

Courchesne et al. (2011) quantitated the total number of neurons in the dorsolateral and mesial prefrontal cortex from seven children with autism and six controls. Autistic children had 67 % more neurons as compared to controls. An interesting

observation by the authors was that autistic patients had more neurons than predicted from the large brain weights. Studies focusing on cortical modularity have attempted to explain findings of increased neuronal density based on the presence of supernumerary minicolumns.

2.4 Minicolumnar Findings

The best-known architectural motif of the cortex is its lamination. However, a vertical organization has also been recognized both anatomically and physiologically. Several anatomical elements have been used to describe morphometric features of the vertical organization. These anatomical elements include pyramidal cell arrays, dendritic bundles, axonal bundles, and the location of double bouquet cells (Casanova 2007). These elements can be used interchangeably as studies have shown their correspondence to each other (Casanova 2008). The most often used method for studying minicolumnarity employs pyramidal cell arrays. Processing conditions, thickness, and staining that allow visualization of pyramidal cell arrays are well known and can be obtained from the classical studies of the Vogts and Yakovlev.

Minicolumns, as defined by pyramidal cell arrays, vary in thickness from 25 to about 55 μm depending on brain region (DeFelipe 2005). They usually have some 80–100 cellular elements spanning layers II through VI. Studies by the Hungarian anatomist Szentágothai showed a preferred placement for pyramidal cells to be located at the center of the minicolumn with interneurons at its periphery forming a so-called shower curtain of inhibition (Szentágothai and Arbib 1975). The dimension of the core space of minicolumns seems conserved among multiple species. Variability in width throughout evolution is primarily ingrained within the outer peripheral space, a compartment housing inhibitory anatomical elements (Casanova et al. 2009a).

In the first study of minicolumnarity in autism, Casanova et al. (2002c) surveyed the morphometry of these modular structures in nine subjects and an equal number of controls. Photomicrographs were taken of Brodmann areas 9, 21, and 22 and studied by computerized image analysis (Buxhoeveden et al. 2000). The algorithm used had been tested against physiologically derived measurements, by 3D modeling and scatter (cell translation around the main axis of the minicolumn), to correct for curvature in case a flat face of a gyrus was not obtainable. The results showed significant reduction in the width of minicolumns primarily attributable to loss within their peripheral neuropil space. The same series was later on analyzed by using a different algorithm, the gray level index (GLI), modified from the method developed by Schleicher and colleagues (Schlaug et al. 1995; Casanova et al. 2002b). The original finding of diminished minicolumnar width was validated by the GLI method. These and other studies have found minicolumnar abnormalities as being widely distributed, but affecting principally, and most severely, the frontal lobes (Casanova 2006; Casanova et al. 2006a).

The minicolumnar findings appear to be quite specific being absent when correcting for mental retardation as in the case of Down syndrome. The morphometric findings also differ from those of other conditions expressing autistic-like behaviors, e.g., rubella babies and tuberous sclerosis. The only condition of similar neuronomorphometry is Asperger where differences are of degree rather than kind (Casanova et al. 2002a). In this regard, the two brain specimens of Asperger individuals examined by Casanova et al. (2002a) gave similar findings to those of autistic subjects but were less severely affected.

In a study sponsored by the Autism Tissue Program, an international group of researchers attempted to reproduce the minicolumnar findings (Casanova et al. 2006b). Different individuals were in charge of various parts of the analysis including patient/tissue selection, photomicrography, computerized image analysis, and statistical analysis of the results. The analysis was performed blind to diagnosis from coded slides. Results were provided to a third party in order to break the blind and perform the preliminary analysis. The initial results, based on an algorithm of the Euclidean minimum spanning tree, were corroborated by using the GLI method. Minicolumnar width, measured as tangential distances between pyramidal cell arrays, was significantly narrower in autistic individuals. In addition, a Delaunay triangulation was implemented to determine the distribution of distances between pyramidal cells (interneurons were thresholded based on size). No significant differences were noted in intracolumnar distances; rather, the results indicated reduced intercolumnar widths. The authors concluded that the total number of pyramidal cells per minicolumn was the same in both the autistic and control groups but that there were an increased number of these modular structures in autism. Finally, reduced measurements of pyramidal cell size as well as their nucleoli suggested a bias in connectivity favoring short axons vs. longer projections. The smaller neurons observed in this study are best suited at maintaining short connections of the type observed in arcuate fibers.

Minicolumnar width reduction in autism spans supragranular, granular, and infragranular layers (Casanova et al. 2010). The most parsimonious explanation to the findings is the possible abnormality of an anatomical element in common to all layers. Compartmentalization of the minicolumn (i.e., studying peripheral neuropil vs. core space) in autism has shown the largest width reduction in its peripheral compartment. This space provides, among others, for inhibitory elements: the so-called shower of inhibition to the minicolumn (see above). The findings have prompted the possibility of an inhibitory/excitatory imbalance in autism and a possible explanation to the multifocal seizures often observed in this condition (Casanova et al. 2003). Casanova has suggested that in autism there is an environmental factor that forces mitosis of periventricular germinal cells in susceptible individuals (Casanova 2012). The migration of daughter cells from the ventricular zone to the cortex then occurs at an inappropriate time when the radially migrating cells (pyramidal neurons) are not integrated with tangentially derived interneurons (see the triple-hit hypothesis at the beginning of the chapter). The end result is an inhibitory excitatory imbalance causing abnormalities in the flow of information through the minicolumn.

It thus appears that the periventricular germinal cells offer a *locus minoris resistentiae* to the expression of pathology in autism. Heterochronic periventricular cell divisions provide for nodular heterotopias and similar migratory abnormalities within the white matter. These changes resemble those observed in tuberous sclerosis, a condition that exhibits marked comorbidity with autism. Viruses that provide an autism phenotype either exhibit neurotropism for periventricular germinal cells or cause cystic damage within the same. Similarly, extreme prematurity is a major risk factor for autism that is usually associated to germinal cell hemorrhages.

2.5 Summary

In comparison with the rest of the literature on autism, few articles have been published on the subject of neuropathology. Given the limited resources, it is unsurprising to find that only 40 or so cases have so far been studied and reported. Characteristically, these reports describe a lack of gross findings and acute changes. The blood–brain barrier appears to be intact. There is no evidence of contusions, hemorrhage, or edema. Although extremely large brain weights have been reported for some autopsied specimens (e.g., more than 1,800 g), this probably represents postmortem edema wherein the fresh postmortem tissue enters in contact with a hyposmolar solution. Microscopic examination in some of these cases shows corresponding evidence of edema not found in properly preserved cases. These specimens need to be eliminated when acquiring a series for quantitative morphometric studies.

Although neuropathological studies suggest a large number of positive findings, few have been corroborated in independent populations. Among the more reproducible findings is Purkinje cell loss. However, the coexistence of Purkinje cell loss with acute reactive gliosis indicates an ongoing process rather than a neurodevelopmental one. Other positive findings like neuroinflammation need to be studied by controlling comparison series for agonal and preagonal conditions. Otherwise astrocytic and microglial activation, primarily affecting the white matter, is expected from specimens suffering from ischemia-reperfusion injury. A significant percentage of autism donor specimens in brain banks have suffered from ischemia-reperfusion injuries during their agonal state. This lesion characterizes the way the patients died rather than the core pathology of the condition.

In the field of neuropathology, findings bear importance when they have explanatory as well as predictive abilities. Certain gross and microscopic findings appear well established, e.g., larger brain size on average, smaller corpus callosum, and heterotopias. It should be clear that the importance of additional findings depends on how much they help explain the neuropathological phenotype already ascertained. Furthermore, the element of predictability should help assign importance to any new findings. We should always ask ourselves, what do the findings help us explain that wasn't previously known?

Autism is a neurodevelopmental condition whose symptoms denote abnormalities of the gray matter. The involved higher cognitive processes and seizures in a

significant portion of patients suggest a cortical localization. Described minicolumnar abnormalities comply with this location. Since supernumerary minicolumns are the mechanism of corticogenesis, their increased number could help explain abnormalities of brain volume as well as connectivity. Longer projections require an increased metabolic load and attendant time delays in transmission; new minicolumns are selectively pressured to provide a small-world network biasing connectivity within networks towards shorter projections. Emergence of this topology optimizes connectedness while minimizing wiring costs within networks. Desynchronization of maturing excitatory (pyramidal cells) and inhibitory (interneurons) elements helps explain the presence of seizures and other higher cognitive impairments in autism.

Postmortem Brain Imaging

By Jane Pickett, Ph.D.

The application of MR imaging to the postmortem brain closes the gap between the macroscopic and microscopic view of this complicated organ (Schumann and Nordahl 2011). A pioneering study comparing postmortem brain MRI and histology "slices" of the same brains showed the direct relationship between atrophy of the hippocampal formation and neuronal loss in Alzheimer's disease (Bobinski et al. 1999). This seemingly simplistic explanation of both ante- and postmortem imaged volume loss in Alzheimer's belies the numerous possible explanations (variable shrinkage, reduction in neuronal volume, etc.) instead of actual reduction in cell numbers found using unbiased stereology.

In autism research, classical neuropathology techniques were augmented by a new postmortem MRI protocol designed to give a three-dimensional representation of the intact, formalin-fixed brain (Schumann et al. 2001). Improvising on a protocol to optimize the *imaging* parameters for postmortem MRI (Blamire et al. 1999), this technique applied a proton density-weighted imaging sequence for optimal differences in gray and white matter contrast in fixed whole brains or hemispheres shown in Fig. 2.1.

The MRI data on the first 39 scanned brains (23 autism and 16 unaffected control) were used in proof-of-principle experiments using a new shape analysis of cerebral white matter gyrifications to classify autistic and control brains (El-Baz et al. 2007; Fahmi et al. 2007). MRI scan data records were made available on an FTP site at the UC Davis MIND Institute from 2001 to 2005 when they were integrated into the informatics platform of the Autism Speaks' Autism Tissue Program (http://www.autismtissueprogram.org).

A Resource for Science: Autism Tissue Program MRI Records

Autism Speaks, an organization dedicated to autism research, is likewise dedicated to the investigation of postmortem brains as a fundamental part of autism research. Its Autism Tissue Program (ATP) has advocated and supported brain donation for research since 1998. MRI DICOM data is stored on the ATP informatics portal (http://www.atpportal.org). The records are accessible to researchers internationally

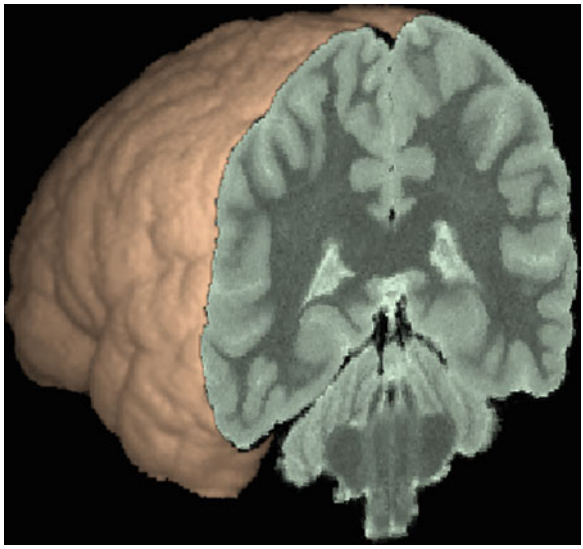

Fig. 2.1 3D reconstruction of MRI data from scan of the whole postmortem brain (Image courtesy of Cindi Schumann, M.I.N.D. Institute, University of California at Davis)

and federated with other autism research programs via the National Database for Autism Research (NDAR, http://www.ndar.org).

Beginning in 2005, formalin-fixed brains were routinely transferred from brain banks affiliated with the ATP to the New York Institute for Basic Research for a specialized neuropathologic examination that begins with MRI and often DTI scanning. A parallel process of MRI, DTI, and neuropathological examination was started on brain specimens in the brain bank for autism in the UK (BBA and RDR) based in Oxford. The imaging data records provide a permanent reference for brains that are further processed and dissected for distribution to many investigators exploring many brain regions—operations that are also tracked on the portal.

By 2012 there were MRI records on the ATP portal representing 63 donors (39 had a diagnosis of autism spectrum disorder (ASD), 20 are unaffected individuals, and others represented related disorders like tuberous sclerosis and chromosome 15q duplication). Antemortem scan records are rarely available; there is one case with two antemortem scan records (age 8, 11) and a postmortem scan record of the donor at age 15.

Diffusion Tensor Imaging (DTI)

In addition to conventional images that depict the macroscopic structure of the brain based on tissue types, "diffusion-weighted" MRI can be used to define the white matter tracts that provide the major connections in the brain (Miller et al. 2011, 2012). This technique faces considerable challenges in postmortem tissue. First, the diffusivity is lower in fixed brains at room temperature. Furthermore, any degradation of the tissue, or air pockets in the sample, can lead to signal distortions.

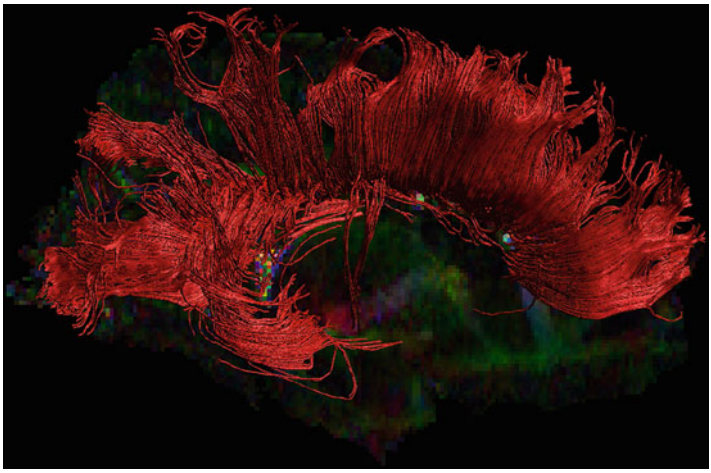

Fig. 2.2 Tractography generated by image analysis of a postmortem brain of a donor with autism shows tracts of the corpus callosum (Image courtesy of Derek Jonest, Cardiff)

Finally, the long-scan times place demands on the stability of the scanner hardware. However, with appropriate alterations to data acquisition and analysis, the virtual reconstruction of tracts is possible. Figure 2.2 shows the corpus callosum of an ASD brain. Connectivity in the ASD brain is a current topic of discussion, and this technology will contribute important information about the integrity of these of interhemispheric connections.

The combination of brain scanning and postmortem tissue dissection enables validation of imaging measurements against the original tissue at the microscopic scale. There is uncertainty about the underlying microanatomical basis of many aspects of the MRI signal, and some of these may be especially relevant to autism research. For example, anisotropy of the diffusion signal in the cerebral cortex (see Fig. 2.3) may relate to the density of cells, myelinated axon bundles, dendrites, and the microscopic minicolumn structure in the cortex.

Summary and New Imaging Frontiers

Postmortem (sometimes called “ex vivo”) scanning means that anatomical images and data can be preserved and shared again and again, even while the actual physical brains are gradually dissected and used by those researching the brain tissue itself. These scans can be reanalyzed as new analysis methods arise in the future.

The promise of diagnostic specificity using an imaging approach validated by histological methods has led to a growing literature where imaging is incorporated into brain banking protocols. It has even been suggested that postmortem imaging can replace autopsies to determine cause of death. This possibility was tested in the UK due to public aversion to autopsies. Roberts et al. (2012) used whole-body CT and MRI followed by full autopsy on 182 cases and compared findings with coroner

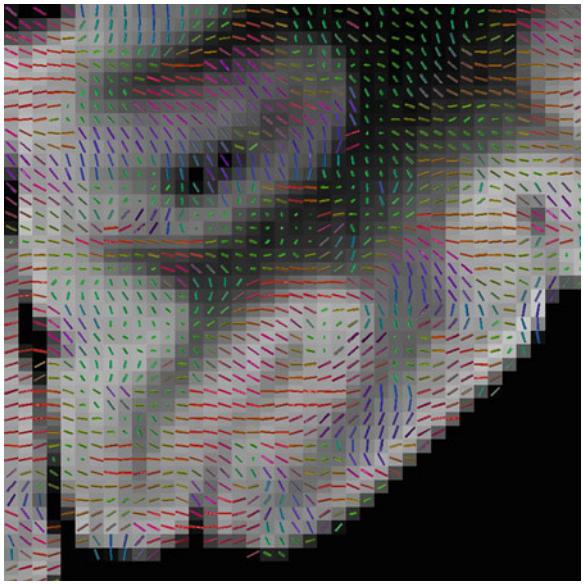

Fig. 2.3 Image of diffusion signal in the cortex of postmortem brain (Image courtesy of Karla Miller, Rebecca McKavanagh, and Steven Chance, all of Oxford University)

reports. They concluded that "The error rate when radiologists provided a confident cause of death was similar to that for clinical death certificates, and could therefore be acceptable for medico-legal purposes. However, common causes of sudden death are frequently missed on CT and MRI, and, unless these weaknesses are addressed, systematic errors in mortality statistics would result if imaging were to replace conventional autopsy."

Imaging of fixed brains should be part of the autopsy process at least as a permanent record of the very rare autism donors and available as an open source when possible. It is unlikely that such a complicated structure can be adequately depicted with imaging alone. On the other hand, new chemical engineering technology called CLARITY (*C*ross-scale and *L*ocally-precise *A*natomy, wi*R*ing, and *I*mmunophenotype in *T*issue H*y*drogel) might bring the field closer to deconstruction without "disassembly" (Deisseroth 2012).

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Chapter 3
QEEG-Guided Neurofeedback for Autism: Clinical Observations and Outcomes

Michael Linden and Jay Gunkelman

3.1 QEEG-Guided Neurofeedback

During the more than 40 year history of EEG biofeedback, now also called neurofeedback (NF), the approach has been used clinically to address attentional problems in attention deficit-hyperactivity disorder (ADHD). Initially, NF was based on the theta/beta ratio, which was measured with eyes open, at the vertex, or the Cz electrode in the International 10–20 Electrode placement system. Generally, the early NF work was based on enhancing beta and reducing the slower theta content (Monastra et al. 1999).

In a review article of NF studies with ADHD spanning 1976-2004, NF provided clients who learn the control of the EEG using NF improvements in hyperactivity, attentional control, impulsivity, and even improved IQ scores (Monastra et al. 2005). This was also confirmed in a more recent meta-analysis of NF in ADHD applications (Arns et al. 2009).

The efficacy of NF in ADHD is now considered well established based on the peer-reviewed published research studies. The efficacy is based on the design characteristics and predictive power of the studies reviewed, including features like the use of matched controls, randomization into treatment condition, independent replication, and improvement in both behavioral and physiological measures. The conclusion was not merely that NF was effective at treating ADHD, but based on the studies' effect sizes, it was a more powerful intervention than medications were.

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However, most of the NF studies these conclusions were based upon did not use a full QEEG to guide the intervention, but were either based on a standard protocol or based on single-channel ratio-based metrics. Interestingly, there is much less literature support for QEEG-guided protocols than for behaviorally or symptom-based approaches.

3.2 Epilepsy and NF

NF applications for epilepsy have a long and well-proven efficacy since the 1960's showing that NF can reduce and occasionally eliminate epileptiform activity in the EEG and the convulsions seen behaviorally. These positive outcomes are seen even in intractable epilepsy where medications have not proven effective (Sterman et al. 1974; Kotchoubey et al. 2001).

In epileptic clients, the literature is more supportive of the use of a full 19-electrode EEGs and even QEEG analysis, with most of Sterman's more recent human research based on the full EEG/QEEG. The American Academy of Neurology and the American Clinical Neurophysiology Society both support the use of QEEG analysis of the EEG in epilepsy and evaluation of epileptiform discharges, including spike dipole analysis and spectral analysis (Nuwer 1997).

Generally in NF the spectral features of the epileptiform discharges are targeted for suppression, with either sensorimotor rhythm (SMR) or slow cortical potential (SCP) based NF training done (Sterman 2000).

3.3 PDD/Autism Treatment Emerges from Attention and Epilepsy Success

In NF there are many who use the technique to help normalize EEG features and will apply the NF experimentally to many disorders, and some practitioners do not even refer to diagnostic issues, but rather are oriented to EEG optimization without the pejorative of a diagnosis.

In autistic spectrum disorders (ASD) and pervasive developmental disorders (PDD), attentional and hyperactivity complaints are common, and the incidence of paroxysmal "epileptiform" discharges in the EEG is estimated at over 40 % conservatively (Gabis et al. 2005), with some suggesting even higher rates of paroxysmal activity. Given the history of success in self-regulation for clients who have epileptiform activity, as well as attentional regulation, many have tried to work with PDD/ASD, as reviewed recently (Coben et al. 2010; Haines and Colletti 2012).

The NF treatment is not specific to autism, but rather oriented to optimizing the brain function each client already has, whether it presents with epileptiform content or rhythmic alterations more like ADHD, anxiety or learning disabilities. The ultimate goal of applying NF to ASD is to improve brain functioning while minimizing side effects. Improvement in brain function can lead to easier success with other therapies, such as those approaches that focus on speech, aggressive behavior, and social skills.

If we use an appropriately conservative perspective with respect to efficacy claims, then NF must be seen as an emerging application, not an established technique for treating ASD with a proven efficacy literature. This is especially true if you use the efficacy criteria adopted by the NF field. These newer emerging applications obviously require further research, with stronger research designs, before claims of actual clinical efficacy can be made (La Vaque et al. 2002).

Even with the conservative perspective held by many in the field, clinical interest in the use of neurofeedback for ASD has been heightened by several case series and some small studies which all showed very promising results (Jarusiewicz 2002; Coben and Hudspeth 2006; Coben et al. 2010; Thompson et al. 2010). Linden is one of the primary investigators in a current study comparing QEEG-guided versus standard NF with students with ASD. Linden's research over the past decade has measured the effects of NF on not only QEEG measures, but intelligence (IQ), attention, hyperactivity and diffuse tensor imaging (DTI), a structural measure of connectivity.

3.4 QEEG-Guided NF for Autism Spectrum Disorder

To understand the QEEG-guided NF approach that we recommend for ASD clients, it is important to first recognize that the practice of NF has evolved dramatically over the past 40 years, as stated above. In the early days, NF was based on symptoms alone, without QEEG guidance. This symptom-driven protocol approach was fraught with problems, including unexpected session outcomes, iatrogenic effects in clients, and protocol redesigns that often appeared to be merely random second-guessing.

Given the variance seen in the underlying pathophysiology of ASD clients, it seems rational to expect that any treatment guided by nothing more than symptomatology will eventually be problematic. QEEG subtype analysis and well-designed NF interventions resolved many of these problems. This modern approach to NF in ASD based on measurements of bioelectrical behaviors matches well with cortical areas of the brain that correspond to the behavioral mechanisms seen in most developmental disorders.

Importantly, it became apparent to those looking at the QEEG in autism that there were many different "subgroups" of EEG findings, rather than a single underlying EEG presentation. This heterogeneity is especially true for the complex spectrum of clinical findings often referred to as the "autisms." In more recent years, researchers and clinicians have begun to develop a system of doing NF protocols based on genetically correlated clusters of EEG findings.

Gunkelman had hypothesized that these EEG clusters might be based on underlying genetics and these resultant endophenotype clusters will respond as a group to specific medications and/or to specific NF interventions (Johnstone et al. 2005).

The therapeutic approach which will provide efficacious intervention is predicted by the endophenotype(s) which the client manifests. Thus, the EEG phenotype selects the protocol and this protocol prediction system enhances the clinical outcome.

3.5 Subtypes or Endophenotypes

The EEG/QEEG can be used to identify the endophenotype(s) involved in any individual's EEG. There is a high inter-rater reliability, generally over 90 % concordance in untrained raters. There are a limited number of phenotypes (11), and they predict almost all of the variance in the EEG (Johnstone et al. 2005). The original phenotype paper was based on retrospective modeling, though the model now has prospective validation done in both medication prediction, such as predicting stimulant efficacy in ADHD, and NF outcomes, as seen in the current QEEG-guided NF studies (Arns et al. 2009). These EEG phenotypes predict effective treatment approaches independent of the DSM diagnosis, as seen in the phenotype paper on addiction treatment outcomes (Gunkelman and Cripe 2008).

Others also evaluated EEG subgroups associated with clinical DSM groupings. Chabot and others at NYU (Chabot et al. 1996) first identified two EEG-based subtypes in children with ADHD: (1) excess theta and (2) excess alpha. These subgroups predicted medication efficacy. In later work they also added "excessive beta" as they broke the initial two groupings into even more subgroups. Interestingly, in our experience this beta subtype one of the most common subtypes present in those with ASD, usually does not respond well to stimulant medications, or to NF protocols which are stimulating, as predicted in the phenotype model.

John and Prichep at NYU have also done cluster analysis in DSM groupings, gaining insight into the pathophysiology of various conditions, such as obsessive-compulsive disorder (OCD). In the DSM, there is only one form of OCD. Cluster analysis found a slow cluster which was not SSRI responsive and an alpha cluster that was SSRI responsive. Gunkelman has seen beta spindles as another cluster, and this group has a negative response to SSRI, not merely a lack of clinical response. The neurometric approach was also used to identify clusters in normal population within the Nx-link database of normal subjects developed by John and Prichep. A database of normal subjects is comprised of individuals with all phenotypes mixed into a grand average of the groups.

Identifying clusters in ADHD was also done by Chabot and Prichep, when they analyzed their approximately 400 ADHD clients from earlier work (Chabot et al. 1996). In their later work, they found 11 clusters within the heterogeneous ADHD clinical group. More recently, we found that the EEG clusters predicted medication response in ADHD to stimulants (Arns et al. 2008), with the slower frontal cluster responding to dopamine reuptake inhibitors.

3.5.1 Endophenotypes Seen in Autism

Early in the application of NF to the autisms, a series of cases was passed through a single EEG laboratory. The presence of a variety of clusters was identified by Gunkelman, and this was discussed with others in the field, including Linden. The phenotypic clusters were initially only observed as general groupings, and only later

was their rate or incidence actually estimated and presented in talks and workshops between 2004 to 2013 at various scientific meetings. Linden's current research at UC San Diego is gathering additional data on these endophenotypes' prevalences.

The presence of slow EEG activity, frequently delta activity, was commonly reported in autism, as also seen in some with learning disabilities. This makes rational sense when seen as evidence of white matter disturbance(s), commonly observed in many clients with an autism spectrum diagnosis. More recently, researchers have seen white matter disturbances with DTI that provides better images of white matter than a static MRI scan (Groen et al. 2011). In Linden's clinical experience, ASD individuals, especially those younger having abnormally high delta activity, often were very active, impulsive, and at times aggressive.

Epileptiform paroxysms are common in autisms, as noted earlier in this chapter. In our experience the distribution of the EEG spectral disturbance often correlates directly with the clinical presentation. Left hemispheric involvement is more likely to involve language disturbances, and if the discharges are seen within the right hemisphere, then a more Asperger syndrome-related presentation is more likely clinically. Frontal discharges often disturb the higher functions of attentional and affective regulation and more posterior and parietal discharges involving disturbances of sensory processing.

Beta spindles are very common in autism, and as classically seen in EEG since initially described by Frederic Gibbs (Stone and Hughes 1990), they represent an easily kindled or irritable cortex with a lower threshold for discharges. This may be seen as sensory hypersensitivity with beta spindles present more posteriorly and parietally in the sensory cortex, though other symptoms appropriate to the cortical regions involved are also seen, such as behavioral explosiveness and difficulty with emotional gating with a right frontal beta spindle distribution. According to Linden's clinical experience and research, this endophenotype pattern often correlates with anxious, overfocused, perseverative and obsessive behaviors.

Temporal changes such as slower content or alpha which suggest a local disturbance may impact language function on the left as well as verbal memory function. The right temporal changes are associated with spatial, prosodic, and nonverbal comprehension and memory functions, such as facial expression, body language, and other emotional contextual perception and comprehension tasks; this right temporal emphasis is commonly seen in individuals with Asperger syndrome. Auditory cortex is deeply embedded temporally at the temporal-parietal junction, and occasionally these areas may also be involved associated with temporal findings.

Mu rhythm (Mu) is seen centrally in a disproportionate percentage of clients with autism, estimated as high as 70 % (Pineda et al. 2008). In Pineda's work, Mu is seen as an effect of a fronto-central disconnection associated with the mirror neuron system. When there are mirroring behaviors, Mu desynchronizes and is not seen in the spectral displays in the EEG waveforms. Though Mu is not considered evidence of any neurologically specific issues such as a lesion, demyelination, and vascular issue, it does suggest a functional disturbance. As classically observed in EEG, Mu is eliminated with even the intent to move.

Low-voltage slow EEGs are seen in a minority of those with autism, and though not specific, in EEG the finding is classically associated with toxic or metabolic encephalopathies, and these clients seem to respond well to medical management such as with methyl-B12, chelation and hyperbaric oxygen and rarely even have been seen to have thyroid or immune system disturbances (Neubrander et al. 2012). Theoretically, this low voltage may be related to environmental factors such as vaccines, pollution, and pesticides.

Coherence changes have been seen in autism, suggesting possible connectivity issues, and these also appear to reflect the symptoms of the client with fronto-central changes associated with the findings of Mu and the right and left temporal changes reflecting language or emotional comprehension presentations clinically. Both hypercoherence and hypocoherence may be observed.

3.5.2 The Incidence of Phenotypes

Recently, we used EEG/QEEG to estimate the prevalence of these subtypes in children with autism. In our experience, the excess beta spindling phenotype or subtype is the most common (70 %). The beta spindles seen in the phenotype model are identified both visually and in spectral analysis. Coherence changes are also commonly observed (70 %). Paroxysmally abnormal EEGs are seen in about 40 % of cases, with epileptiform spike activity more common than many may assume merely by looking at the incidence of convulsions in this population. As mentioned, the excessive slow content is common, with an estimated incidence of 30 % in autism. The low-voltage slow pattern suggesting toxic or metabolic issues comprises only 10 % of the cases in our population.

Coben and colleagues (2012) recently showed five "subtypes" in the cases they researched in autism. They used relative EEG power and looked at 91 individuals on the autism spectrum and 310 normal controls. They report excesses of beta and alpha in about one-fourth of the ASD sample (26.5 % and 25.3 %); they have also described subtype patterns of coherence or connectivity.

3.6 Predictive Validity

Obviously, EEG patterns are not simplistic, and linear models of real brain function are not even close to a proper reflection of reality. Even with our EEG-based endophenotype approach, more than one pattern is commonly evident. The search for a single biomarker in the ASD is no longer a realistic expectation. In the presence of a variety of findings, the important feature of any model explaining the observed findings is that it must have some predictive validity. The model should at least predictively correlate with symptoms and preferably also with the proper treatment approach to deal with the symptoms and their underlying neurophysiology.

On a case basis, the EEG phenotypes seem to correlate well with each individual's clinical presentation, and even though these phenotypic clusters cut across the DSMIV-TR categories and are not considered diagnostically specific, this phenotype framework can be used to guide a personalized approach to medicine or NF through its ability to predict treatment responses (Johnstone et al. 2005; Gunkelman 2006; Arns et al. 2008).

The phenotype model was tested in ADHD with the goal of predicting stimulant medication efficacy. The phenotype model was shown to be predictive of effective response for stimulant medication in the children, with 49 ADHD subjects studied against 49 matched controls (Arns et al. 2008). The phenotype approach has also been used in other DSM applications effectively to predict effective treatment approach.

3.7 QEEG-Guided Neurofeedback

QEEG-guided NF normalizes poorly regulated brain regions that are the neural representation of specific clinical presentations (Arns et al. 2008, 2009). With ASD, this means that the treatment approach is personalized to match each individual's phenotypic pattern(s) and clinical presentation. The goal of the initial NF with ASD is to correct amplitude abnormalities and balance brain functioning. Following these initial interventions, many of the coherence findings will have normalized, though some areas may remain either hyper- or hypocoherent. These remaining findings, which were resistant to initial interventions, are then subjected to coherence neurofeedback, which is intended to improve the connectivity and plasticity between brain regions where residual changes in coherence remain.

When treating clients with conditions as heterogeneous as autism, obviously an EEG/QEEG baseline is required to properly designing a personalized NF treatment plan. The QEEG identifies a client's phenotype pattern(s). Using those patterns to guide subsequent neurofeedback or medication management, it becomes possible to develop a customized NF treatment approach that normalizes and optimizes each individual's EEG.

These tailored interventions have protocol-specific effects, such as left temporal lobe interventions affecting speech and language communication, right temporal interventions affecting social and emotional functions, and frontal interventions influencing attention, and central and posterior abnormalities can influence sensory and motor functions. Our specific outcomes clinically include significant speech and communication improvement, less aggressive behavior, calmer demeanor, increased attention, improved eye contact, and increased socialization. Many of our clients have generally been able to reduce or eliminate their medications following completion of NF based on the phenotype model, with the exception of anticonvulsant medication in some with residual paroxysms. This is not unexpected because currently there is no medication that has been specifically developed for ASD.

3.8 Not All *Z*-Score Outliers Are Abnormal

EEG results are compared with a normative reference population to evaluate which measured values differ from the mean values. Due to the plethora of statistical comparisons done when processing an EEG through the QEEG databases, it is highly likely that divergence from the mean will be seen merely due to multiple statistical comparisons. This is especially true as the categories that we evaluate quantitatively increase: now including not merely absolute and relative power and connectivity but also bursts metrics, multivariate analysis, and many other features. With this increase in statistical manipulation and lack of correction for these rapidly expanding number of metrics, when we see a divergence it is most important to focus on the validity of any given divergence, as the statistical likelihood of a random outlier has dramatically increased. Patterns of deviation are needed when correction for multiple comparison is not performed in order to assure any observed deviation is a real outlier and not due to the statistical manipulations.

Aside from the reliability of the EEG sampled for analysis, the underlying validity of the findings is also critical. Although a statistical divergence may be associated with an actually abnormal finding, there are two other possibilities. Divergent values also may be due to:

1. A compensatory mechanism that helps the brain with another abnormal feature
2. A unique skill or performance state that is not compensatory for any other finding (such as very fast alpha and superior declarative memory performance or EEG changes associated with meditation)

3.9 CNS Arousal and Frequency "Tuning"

Databases are not very adept at describing divergence when the usual banded activity shifts outside an expected frequency range. There are multiple statistical divergences seen due to frequency drifting outside normally expected ranges. As an example, a normal amount of power and coherence seen as a normal pattern of alpha, if merely slowed to 7 Hz without coherence or power alterations, will be seen as excessive theta (not slowed alpha) and as hypercoherent (when coherence was not altered), merely due to the database's expectation of the alpha power and coherence pattern at a higher frequency range. The database will not say that this is a frequency issue but that the coherence and power pattern is in a normative range. The databases will say the content is hypercoherent and that there is excessive power in theta. In this case, the statistical divergences in coherence and power would be distractions from the real task of speeding up the 7 Hz slowed alpha activity.

Shifts in the underlying frequency tuning in the EEG are described as a phenomenon called "brain-rate." This term is coined and mathematically defined by Pop-Jordanov of the Macedonian Academy of Science and Art (Pop-Jordanova and Pop-Jordanov 2005). The frequency shifts are associated with variations in the CNS arousal level.

These frequency-shift-related statistical divergences which are not meaningful may even be directly a distraction from the real issue. This shows that for the clinician the important task is to track both clinical and behavioral changes during training and correlate these with the EEG/QEEG findings. The clinician's oversight assures that the features being normalized with neurofeedback were actually more than just statistical outliers and that the findings are not merely compensatory, in which case the client's presentation would worsen with the neurofeedback.

The use of QEEG-based NF with ASD is becoming a highly personalized and apparently successful treatment option to address the behaviors we see impacted by these disorders, and this approach continues to be very promising to deal with undiagnosed epileptiform activity, speech and communication, aggressive behavior, irritability, poor attentional skills, poor eye contact, and impaired socialization that comprise much of autism spectrum's clinical presentation.

The addition of QEEG-directed NF to the clinical armamentarium has given a significant percentage of our patients the ability to begin moving on the road to recovery, and improvements are seen in the majority of clients with ASD. Many of them have gone much farther than they would have ever been expected to with the other treatments available. This is especially true if we were without the insight into the client's pathophysiology associated with their individual presentation which the QEEG provides. This is especially clear with the identification of epileptiform findings when they are unexpected due to absence of behavioral convulsion.

Cases with episodes of epileptiform "subclinical seizures," if and when identified, suggest a clinical trial of anticonvulsant medication or appropriate NF, even with cases that do not have a history of convulsive seizure activity. This would never be the case without the insight the EEG/QEEG testing provides. Historically, only children with documented convulsive activity are prescribed anticonvulsants. The approximate 40 % of cases with autism which have epileptiform content would seldom have received appropriate anticonvulsant medication without these findings. The use of anticonvulsant medication is becoming more accepted for children on the autism spectrum who do not have convulsions but who have paroxysmal EEG brain wave activity to at some point be given a clinical trial of anticonvulsant therapy, especially when other treatments are not producing positive results. It is not uncommon for parents to report that the addition of an anticonvulsant medication or appropriate NF protocol to their child's treatment regimen resulted in increased language, better vigilance, improved attention, cognition, and positive behavioral changes as the EEG function normalizes.

Through the use of EEG/QEEG, we are now more successful in choosing appropriate treatment approaches and protocols which are personalized for each client. Through the knowledge of the client's phenotype(s), we have been able to target specific treatments rather than "blindly prescribing" a clinical approach based on behavior alone, as is commonly done by those psychiatrists and neurologists who do not obtain EEG/QEEG to help guide their work. The long-term goal of neurofeedback with ASD is to improve brain functioning long-term without side effects. This neurological improvement also leads to better success with other treatments and therapies such as speech, behavior, occupational therapy, and social skills.

3.10 Neurofeedback Research with Autism

3.10.1 Pilot and Case Studies

Two pilot group studies of neurofeedback for ASD have been conducted. In the first (Jarusiewicz 2002), twelve children each were assigned to an experimental or a control group. The experimental group received a mean of 36 treatment sessions (range = 20–69). Treatment protocols were based on standard EEG frequencies and locations. The Autism Treatment Evaluation Checklist (ATEC) (Rimland and Edelson 2000) was used to assess outcome. Children who completed NF training attained an average 26 % reduction in the total ATEC rated autism symptoms in contrast to 3 % for the control group. Parents reported improvement in socialization, vocalization, anxiety, schoolwork, tantrum behavior, and sleep habits, while the control group had minimal changes in these domains. However, the outcome measure used is based on only parent report with no other objective measures utilized.

The second pilot study of the effects of neurofeedback was conducted by Kouijzer et al. (2009a, b). Fourteen children with ASD, seven in the treatment and seven in the wait-list (no treatment) control group, were matched for age, gender, and IQ scores, but were not randomly assigned. The treatment group received 40 sessions of neurofeedback on the right sensory motor strip. Theta activity (4–7 Hz) was inhibited, while sensorimotor (SMR) activity (12–15 Hz) was rewarded. Pre- and posttreatment assessment consisted of EEG learning curves, QEEG analyses, tests of executive functioning, and behavior rating scales (CCC-2, Dutch Autism Scale). The findings showed that the neurofeedback-trained group demonstrated significant improvement in attentional control, cognitive flexibility, and goal setting compared to the control group. Results of parent rating scales also showed improvements in social interaction and communication skills. These changes were associated with improvements in EEG learning curves.

Interestingly, this same research group performed a 12-month follow-up of the treated patients with ASD (Kouijzer et al. 2009a). Both changes in executive functioning and behavior were maintained suggesting that neurofeedback may have long-lasting effects for children with autism as it has been shown by Lubar (1991) and Monastra et al. (2005) to have with students with ADHD. These pilot studies have shown positive results, but caution should be exercised as their sample sizes were quite small. Nevertheless, the optimism regarding their findings has led to more controlled research with larger sample sizes.

3.10.2 Controlled Group Studies of Neurofeedback for ASD

In the largest published, controlled study to date of neurofeedback for autistic disorders, Coben and Padolsky (2007) studied 49 children on the autistic spectrum. The experimental group included 37 children that received QEEG-guided neurofeedback

(20 sessions performed twice per week), and the wait-list control group included 12 children that were matched for age, gender, race, handedness, other treatments, and severity of ASD. A broad range of assessments were utilized including parental judgment of outcome, neuropsychological tests, behavior rating scales, QEEG analyses, and infrared imaging. Treatment protocols were assessment based (including QEEG power and coherence) and individualized for each child receiving neurofeedback training with a specific focus on the remediation of connectivity anomalies. Based on parental judgment of outcome, there was an 89 % success rate for neurofeedback and an average 40 % reduction in core ASD symptomatology based on parent rating scales. There were also significant improvements, as compared to the control group, on neuropsychological measures of attention, executive functioning, visual-perceptual processes, and language functions. Reduced cerebral hyperconnectivity was associated with positive clinical outcomes in this population. In all cases of reported improvement in ASD symptomatology, positive outcomes were confirmed by neuropsychological and neurophysiological assessment. The benefit to harm ratio, which is regularly utilized to determine if a treatment is successful and safe, was 91:1, the highest of any treatment for ASD studied to date.

Two studies have focused on abnormal Mu rhythms (previously discussed) (Oberman et al. 2005) in children with autism with neurofeedback. In a series of two experiments, Pineda and colleagues (Pineda et al. 2008) studied 27 children with high-functioning autism. In study 1, eight high-functioning males were randomly assigned to an experimental ($n=5$) or placebo ($n=3$) group. One subject dropped out of the experimental group midway through the training. Neurofeedback training included 30 thirty-minute sessions with rewards for Mu-like activity (8–13 Hz) and inhibits for EMG (30–60 Hz) at C4 (right central location). Parent rating scales (ATEC) (Rimland and Edelson 2000) showed small changes (9–13 %) in two of the four experimental participants. These pilot data should be considered preliminary due to the very small sample size.

In the second study, 19 children with high-functioning ASD were randomly assigned to an experimental ($n=9$) or placebo ($n=10$) group. One very positive addition to this study was the verification of their diagnoses by administering the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 2000) and the Autism Diagnostic Interview–Revised (ADI-R) (Rutter et al. 2003). Neurofeedback training was similar to study one except the reward band was now 10–13 Hz. Parent ratings showed a small but significant reduction in symptoms (ATEC Total score). However, of interest was an increase in ratings of Sensory/Cognitive Awareness in excess of 40 % that did not occur in the placebo control group. According to their parents, participants improved in some areas and worsened in others, and these areas of improvement may be based upon the frequencies and locations trained.

In another study related to Mu rhythms, Coben and Hudspeth (2006) studied fourteen children with ASD who were identified as having significantly high levels of the Mu rhythm activity and a failure to suppress Mu during observational activity. They all received assessment-guided (QEEG guided) neurofeedback, with a strong focus on aspects of Mu power and connectivity. The participants were non-randomly

assigned to an interhemispheric bipolar training ($n=7$) or a coherence training ($n=7$) group designed to increase connectivity between central regions and the peripheral frontal cortex. All patients were given neurobehavioral, neuropsychological testing and QEEG assessment. Both groups of patients improved significantly on neurobehavioral and neuropsychological measures. However, only in the coherence training treatment group was Mu activity significantly reduced. Increased coherence was associated with diminished Mu and improved levels of social functioning.

Coben (2008) conducted a controlled neurofeedback study focused on intervention for prominent social skill deficits based on a facial-/emotional-processing model. Fifty individuals with autism were included in these analyses, and all had previously had some neurofeedback. All patients underwent pre- and post-NF neuropsychological, QEEG, and parent rating scale assessments. Fifty individuals were each assigned equally to an active neurofeedback and wait-list control group, in a nonrandomized fashion. The two groups were matched for age, gender, race, handedness, medication usage, autistic symptom severity, social skill ratings, and visual-perceptual impairment levels. Neurofeedback training was QEEG connectivity guided and included coherence training (along with amplitude inhibits) between maximal sights of hypocoherence over the right posterior hemisphere. The group that received the coherence training showed significant changes in symptoms of autism, social skills, and visual-perceptual abilities such that all improved. Regression analyses showed that changes in visual-perceptual abilities significantly predicted improvements in social skills. QEEG analyses were also significant, showing improvements in connectivity and source localization of brain regions (fusiform gyrus, superior temporal sulcus) associated with enhanced visual/facial/emotional processing.

In the five controlled group studies that have been completed, a total of 180 individuals with autism have been studied with positive results reported in each study. These findings have included positive changes as evidenced by parental report, neuropsychological findings, and changes in the EEG (Coben 2008). Both Coben and Padolsky (2007), Yucha and Montgomery (2008) and Coben, Linden & Meyer (2010) have viewed these data as demonstrating a level of efficacy of possibly efficacious based on the standards put forth by the Association for Applied Psychophysiology and Biofeedback (AAPB). Added to these initial findings of efficacy is preliminary evidence that the effects of neurofeedback on the symptoms of autism are long-lasting (1–2 years) (Kouijzer et al. 2009b; Coben and Myers 2010).

Pineda and Linden are currently conducting research at the University of California at San Diego (UCSD). They are investigating QEEG, fMRI, and DTI results of both QEEG-guided and Mu neurofeedback in both ASD and typical students. ASD and typical students were all treated with 60 h (45 forty-five minute sessions) of neurofeedback. Some of the students received QEEG-guided neurofeedback and the other half Mu suppression NF. The NF for both groups was administered using consistent scripts utilizing Thought Technology software and hardware. Preliminary results are indicating more significant improvements in DTI (volume)

related to connectivity between brain regions for ASD students compared to typical students. These DTI results would support previous research of improved connectivity in students with ASD from NF.

3.10.3 Limitations

While the findings to date are initially encouraging, there are limitations that prevent firm conclusions. First, these studies have largely included nonrandomized samples. It is possible that an unknown selection bias exists which could have impacted the findings. Second, none of these past studies (except the current study at UCSD) have included participants or therapists/experimenters who were blind to the condition. Knowledge of group placement could have impacted the findings such that those in treatment (and their parents) would be prone to report significant changes. Third, there has been no attempt to control for placebo effects, attention effects from a caring professional, or expectations of treatment benefit; however, in the current UCSD study (Pineda and Linden unpublished findings) typical students completed neurofeedback as a control group. A randomized, double-blinded, placebo-controlled study, although complicated and difficult to do, would be optimal to further demonstrate efficacy.

Another unknown is that very young children (less than four years of age) and adults have not been represented, in these studies, so generalization to the current population is not possible. These populations should be also the focus of future research investigations especially because children are currently being diagnosed at before the age of one.

Furthermore, ASD individuals who are lower functioning or who have more severe symptoms associated with autism have not been included in most of the research to date, although clinicians, including the authors, have had successful treatment outcomes. Overall, the use of QEEG to assess subtype patterns of ASD is important in both the diagnosis and treatment selection and success.

3.11 NF Research with ASD Conclusion

Even with the conservative perspective held by many in the field, clinical interest in the use of neurofeedback for ASD has been heightened by several case series and research studies. If an appropriately conservative perspective with respect to efficacy claims is taken, then neurofeedback must be seen as an emerging application. This is especially true if you use the efficacy criteria adopted by the field of neurofeedback. These newer emerging applications obviously require further research, with stronger research designs, before claims of actual clinical efficacy can be made. However, the use of QEEG-guided neurofeedback with ASD is becoming a highly individualized and successful treatment option and continues to be very promising.

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Biography

Michael Linden is a licensed clinical psychologist, marriage and family therapist, and a nationally certified biofeedback and neurofeedback therapist. Dr. Linden has specialized in the assessment and treatment of children, adolescents, and adults with attention deficit disorder (ADD) since 1982 and is the director of the ADD Treatment Centers and Neurofeedback Programs at Mission Psychological Consultants' three locations in San Juan Capistrano, Irvine, and Carlsbad. He has published several studies on qEEG assessment and neurofeedback treatment of ADD and autism over the past several years.

Jay Gunkelman was licensed on qEEG, biofeedback, and neurofeedback in 2002 by B.C.I.A. He began his career in 1972 with the first State Hospital-based biofeedback laboratory, and then specializing in EEG for decades, Jay is one of the most experienced clinical and research EEG/qEEG specialists in the world. He has recorded more 500,000 EEGs. Author of many scientific papers, and a mounting list of books, his depth of understanding of the mind/brain's function is unique. Jay is a popular lecturer worldwide, and he has occupied leadership positions in the field's professional societies, as well as running a successful EEG/qEEG business.

Chapter 4
Event-Related Potential Studies of Cognitive Processing Abnormalities in Autism

Estate M. Sokhadze, Joshua Baruth, Allan Tasman, and Manuel F. Casanova

4.1 Theoretical Models of Autism

Autism is recognized as a pervasive developmental disorder (PDD) usually evident during the first 3 years of life (Ruble and Brown 2003; Volkmar and Pauls 2003). Several neuropsychological models have been proposed to explain the cognitive deficits found in autism spectrum disorders (ASD) (Baron-Cohen 2004), one of which is based on salient deficits in executive function (Burack 1994; Hughes et al. 1994; Ozonoff 1997; Hill 2004). Executive functioning skills fall under the purview of those prefrontal functions that facilitate problem-solving, flexible set-shifting, and forward planning in the implementation of goal-directed behavior (Hughes et al. 1994). The executive deficits in this autism model have been related to specific frontal mechanisms, principally to the prefrontal and midfrontal cortices and

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associated neural circuitries (Bishop 1993; Hill 2004). The domain of executive functions has significant implications for developmental psychopathologies, but there are still doubts (Griffith et al. 1999) regarding the causal explanation of signs and symptoms in autism as stemming exclusively from frontal executive functioning abnormalities.

Other integrative models of autism mainly focus on impaired functional connectivity (Villalobos et al. 2005; Welchew et al. 2005). One of the theoretical models aimed to unify existing knowledge on the etiology of autism focuses on abnormalities in neural connectivity (Belmonte et al. 2004a, b). The model states that autism might be characterized by functional disconnectivity of networks important for specific aspects of social cognition and emotional and behavioral control. Impaired connectivity between the posterior sensory modality-specific attention system and the anterior associative prefrontal cortex could be one of the most important impairments in autism.

Another important model of autism takes a "minicolumnar" perspective (Casanova 2005, 2006) and is based on neuropathological findings. Autism has been associated with cytoarchitectural abnormalities of the minicolumn, a radially oriented assemblage of neurons and other cellular elements considered to be an elemental modular microcircuit of the neocortex (Casanova et al. 2002b, c, 2006a, b). Minicolumns are comprised by a core vertical column of pyramidal cells and their projections surrounded by peripheral neuropil containing GABAergic inhibitory interneurons (Mountcastle 1997; Buxhoeveden and Casanova 2002; DeFelipe 2004). Among these, double-bouquet cells feature axon bundles which provide a "vertical stream of inhibition" (Mountcastle 1997) insulating excitatory flow in the minicolumnar core from the activity of surrounding minicolumns (Favorov and Kelly 1994a, b; DeFelipe 1999, 2004). The value of each minicolumn's output is insulated to a greater or lesser degree from the activity of its neighbors by GABAergic inhibition in its peripheral neuropil space. Peripheral neuropil has been shown to be reduced in postmortem tissue of autistic individuals (Casanova et al. 2002b, c) most prominently over their prefrontal cortex (Casanova et al. 2006a, b). This may provide the basis for overlapping fields of lateral inhibition which interact in a combinatorial manner to influence the excitatory output of each minicolumn in the network.

Consistent with reported neuropathological findings of Casanova et al. (2002b), Rubenstein and Merzenich (2003) have proposed that reduction of GABAergic inhibitory activity could result in hyperexcitability of minicolumnar circuits, while Oblak, Gibbs, and Blatt (2010) found decreased GABA receptors in the cingulate cortex and fusiform gyrus in autism, and these results may explain some symptomatology of autism, including increased incidence of seizures and sensory (e.g., auditory, tactile) hypersensitivity (Casanova et al. 2003). This hypothesis is consistent with findings of reduced minicolumnar peripheral neuropil space in the neocortex of autistic individuals relative to controls (Casanova et al. 2002a, b, c). In this model, a reduction in the peripheral neuropil space would result in smaller minicolumns which would coalesce into discrete, isolated islands of coordinated excitatory activity. These islands hinder the binding of associated cortical areas, arguably promoting focus on particulars as opposed to general features.

4.2 Event-Related Potentials in Cognitive Neuroscience Research

Our review is aimed to explore this putative abnormality of neural and functional connectivity in autism by electrophysiologically measuring performance on cognitive tests with electroencephalographic (EEG) response recording—in particular, event-related potential (ERP) measurements. Analysis of ERP components is one of the most informative dynamic methods of investigation and monitoring of information-processing stages in the human brain. Different amplitude and latency characteristics of ERP waves at specified topographies reflect both early sensory perception processes and higher-level processing including attention, cortical inhibition, memory update, and other cognitive activity (Polich 2007; Duncan et al. 2009). ERPs provide a method of studying cognitive processes in typical subjects and also provide a tool to assess differences in individuals with developmental pathologies. Despite significant advances in functional neuroimaging (e.g., fMRI), the EEG/ERP measures still represents an important tool for brain research in psychiatry, as many psychiatric diseases correlate with certain altered patterns of EEG responses (Lenz et al. 2008). Such neurophysiological EEG/ERP alterations can either serve as valid biological markers for functional diagnostic purposes or for better understanding of the cognitive functions which are disturbed in neurodevelopmental disorders such as ASD.

Event-related potentials reflect the activation of neural structures in sensory cortex, association cortical areas, and brain areas related to higher-order cognitive processes. Important information about cortical activity in autism can be obtained with ERPs, as stimulus-driven electrocortical field potentials, and can be categorized as short-latency (exogenous, e.g., N100) or long-latency (endogenous, e.g., P300) ERPs, which reflect early-stage, modality-specific, and late-stage polymodal associative processing, respectively. It has been assumed that early components (e.g., P100, N100) reflect exogenous processes modulated by the physical attributes of the stimulus (i.e., brightness for visual stimuli, loudness of auditory stimuli, etc.), but not by cognitive processes (Coles and Rugg 1995). However, many studies have shown that attention processes may operate at the early stage (e.g., before 200 ms) and can influence stimulus processing at the later stage (Herrmann and Knight 2001). P100 may reflect a facilitation of early sensory processing of attended stimuli, while N100 may reflect the orienting of attention towards task-relevant target stimuli (Näätänen and Michie 1979; Luck et al. 1990; Hillyard and Anllo-Vento 1998). Posterior visual P100 is generated within the fusiform gyrus (Heinze et al. 1994), whereas N100 is probably generated by distributed dipoles in lateral extrastriate cortex (Gomez Gonzales et al. 1994) with contribution from parieto-occipital and occipitotemporal areas (Yamazaki et al. 2000). Anterior P100 and N100 components occurring within a comparable time window result from frontal generators (Clark et al. 1994).

The most studied endogenous ERP is the P300 (300–500 ms poststimulus). The P300 is obtained in an oddball paradigm, wherein 2 stimuli are presented in a

random order, one of them frequent (standard) and another one rare (target) (Pritchard 1981; Polich 2003). A modification of the task has been used where a third, also infrequent novel distracter is presented along with standard and rare target stimuli. It was reported that these novels elicit a fronto-central P300, so-called P3a, whereas the rare targets elicit a parietally distributed P300, so-called P3b (Katayama and Polich 1998; Polich 2003). The P3a is recorded at the anterior locations and reflects frontal lobe activity (Knight 1984; Friedman et al. 1993). In a three-stimuli oddball task, the P3a is interpreted as "orienting" and the P3b as an index of ability to sustain attention to target. Source localization techniques have claimed that multiple brain areas are involved in the generation of the visual P3b: the hippocampus and parahippocampal areas, the insula, the temporal lobe, occipital cortex, and the thalamus (Rogers et al. 1993; Goto et al. 1996; Mecklinger et al. 1998; Herrmann and Knight 2001). Most studies agree that the P3b has multiple dipole sources (Knight 1997; Halgren et al. 1998; Townsend et al. 2001).

There is a negative endogenous ERP component (N200 or N2b), located over centro-parietal scalp locations and occurring about 180 ms and 320 ms poststimulus (Näätänen et al. 1978, 1993). This component is associated with categorization, perceptual closure, and attention focusing ultimately signaling that a perceptual representation has been formed (Potts et al. 2004). The posterior visual N2b is enhanced if the presented stimulus contains a perceptual feature or attribute defining the target in the task. A frontal positive component (P200, P2a) in a latency range comparable with the posterior N2b (i.e., 180–320 ms poststimulus) has been reported in working memory and attention tasks. The P2a recorded over inferior prefrontal recording sites appears to be selectively responsive to the evaluation of the task relevance of presented visual stimuli, and source localization places dipoles of this component in the orbitofrontal cortex (Potts et al. 1996, 1998). Kenemans et al. (1993) described this frontal positivity as a component that indices the hierarchical selection of task-relevant features for further processing. Information about processes related to response conflict detection and processing, as well as inappropriate response inhibition, can be extracted from the fronto-central ERP component N200 (West 2003; West et al. 2004), which is thought to originate from the anterior cingulate cortex (ACC) and prefrontal sources (Donkers and van Boxtel 2004).

4.3 ERP in Autism

Several studies using visual and auditory modalities in cognitive tasks have shown that children with autism show abnormalities in ERPs (reviewed by Kemner et al. 1999; Bomba and Pang 2004). Children with autism diagnosis have been found to differ from typical children mainly with respect to the P300 (P3b) in standard oddball tasks. Kemner et al. (1994, 1995, 1999) have reported an abnormally small occipital P3b in response to target visual stimuli. Also, deficits in auditory processing in autism as indexed by ERPs have been described by different authors (Oades et al. 1988; Courchesne et al. 1989; Ciesielski et al. 1990; Lincoln et al. 1993; Ferri

et al. 2003; Bertone et al. 2005). In autism the most consistent and frequently reported abnormality is P3b amplitude attenuation with auditory stimulus presentation (Bruneau et al. 1999; Seri et al. 1999; Bomba and Pang 2004). Kemner et al. (1994) reported that the visual N200 to novel distracters is larger when a person with autism is performing a task even when these novel stimuli are not relevant to the task in question. Both the frontal P3a to novel stimuli and the parietal P3b to attended auditory target stimuli were reported to be abnormal in autism (Townsend et al. 2001). However, in a simple visual target detection task, there were no P3b amplitude differences found between autism and typical control subjects (Courchesne et al. 1989; Ciesielski et al. 1990).

Kemner et al. (1994, 1995, 1999) found reduced central and occipital P300 in response to visual stimuli, but these authors also reported that the parietal P3b was larger in autistic children and interpreted their results in terms of differences in attentional resource allocation, as the parietal P3b is more sensitive to such task manipulations as stimulus relevance and probability (Donchin and Coles 1988) and less dependent on modality than the occipital P3b (Kemner et al. 1999). Courchesne et al. (1989) also found a smaller frontal N450 to visual stimuli, but Kemner et al. (1999) could not replicate reduced late negativity (so-called Nc, 400–700 ms post-stimulus). In general, studies using a simple visual target detection, as compared to cross-modal (e.g., audiovisual integration) tasks, have found no significant differences in the P3b to targets in autistic children compared to controls, while abnormalities were present in dissociations of frontal (delayed) and posterior (relatively intact) P300 in visual attention tasks (Townsend et al. 2001).

The frontal novelty P3a is less explored in autism and results are not consistent (Ciesielski et al. 1990, 1995; Townsend et al. 2001; Ferri et al. 2003). It was reported that the frontal P300 (also referred to as P3f, Townsend et al. 2001), which reflects attention orienting, was delayed or missing in autistic subjects, and this finding was interpreted by the authors as a disruption of both parieto-frontal and cerebello-frontal networks critical for efficient cross-modal integration. Our results in a visual three-stimuli oddball with novel distracters (Sokhadze et al. 2010a) also showed prolonged frontal attention-related negativity (e.g., N200) and delayed frontal orienting-related positivities (P2a, P3a) in autism.

Abnormalities in central sensory processing both in auditory and visual modalities have been described by different authors in autism (Verbaten et al. 1991; Kemner et al. 1994, 1999; Ferri et al. 2003; Bomba and Pang 2004). However, most of these studies analyzed and reported P3b (Courchesne et al. 1989; Ciesielski et al. 1990; Lincoln et al. 1993) and P3a (Townsend et al. 2001; Ferri et al. 2003) findings. Notwithstanding the large number of studies published on ERPs in autism, there are only a few papers reporting short-latency ERP components' differences in individuals with autism. Most of the studies outline hyperactivation as well as an abnormal pattern of primary perceptual processes (e.g., low selectivity), abnormal top-down attentional control (e.g., orienting to novelty), and irregular information integration processes (Belmonte and Yurgelun-Todd 2003a, b). In control subjects, the frontal P300 (P3a) occurs earlier and commonly precedes parietal P300 (P3b), but in autistic subjects the P3a and P3b components were found to peak almost at the same time

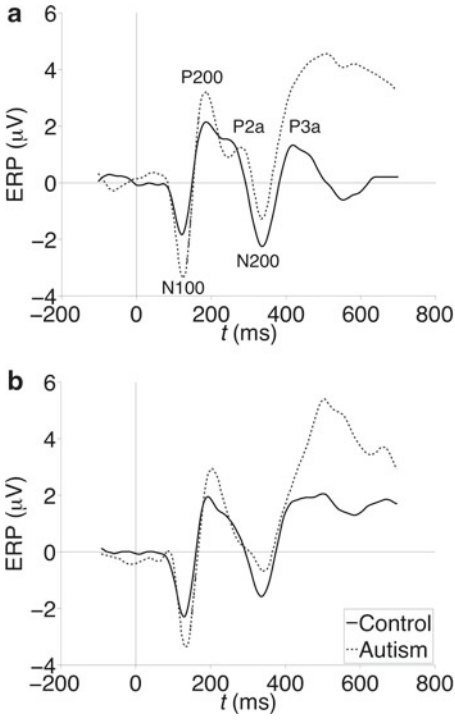

Fig. 4.1 Grand average frontal ERP to target (**a**) and rare nontarget (**b**) stimuli in ASD and control groups ($N = 16$ per group). The ASD group shows more negative N100 component both to targets and nontargets as compared to controls. The ASD group presents higher and delayed P3a peak as compared to controls

over the frontal and parietal sites in a spatial visual attention task (Townsend et al. 2001). In our own study (Sokhadze et al. 2009b), both rare target and novel stimuli elicited a delayed P3a component in the autism group (Fig. 4.1). The latency of this component usually is associated with the speed of attentional orienting to stimulus and reflects prefrontal working memory processes. Posterior P3b indexing context update and closure was also found to be delayed (Fig. 4.2) but was not significantly attenuated in the ASD group (Sokhadze et al. 2009a, b, 2010b).

There is a model of autism that has centered on the construct of "weak central coherence" or the diminished capacity to integrate information into coherent or meaningful wholes (Frith and Happé 1994). This abnormality of global processing refers to an inability to integrate components of perceived patterns to form a whole (Frith and Happé 1994; Happé 1999). Frith and Happé's model assumes superior local processing and weaker integrative processing in autism. Some research groups have questioned this view, reporting evidence of intact global processing in conjunction with superior local processing in autism (Morgan et al. 2003; Mottron et al. 2003; Plaisted et al. 2003; Pellicano et al. 2005). The "weak central coherence" hypothesis was recently supported by Brock and colleagues (2002; Rippon et al. 2007) who proposed that many features of autism could be associated with a reduction of the integration of specialized local neural networks in the brain caused by a deficit of temporal binding between different cortical areas.

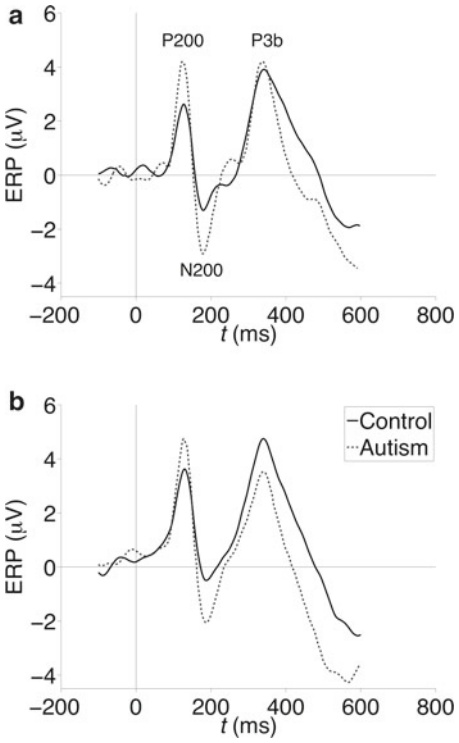

Fig. 4.2 Centro-parietal ERP to target (**a**) and rare nontarget (**b**) stimuli in ASD and control subjects ($N = 16$ per group). The ASD group has higher amplitude of P200 and N200 ERP components as compared to controls

Rippon et al. (2007) recently developed further a hypothesis initially suggested in their earlier papers (Brock et al. 2002; Brown et al. 2005) that a wide range of deficits in autism might be understood by disrupted information integration in the brain. Specifically, the authors argued that neural and cognitive developmental abnormalities result in an imbalance between the processes of specialization and integration (Johnson 1999) and that specialized information-processing units in the brain are effectively disconnected from one another. In this theory, a distinction was made between hypothesized reductions in global connectivity and normal or even increased connectivity within these local networks (Rippon et al. 2007). Several other studies have supported the "functional disconnection" hypothesis, which implicates abnormalities of neural connectivity in autism (Belmonte et al. 2004a, b; Casanova 2005, 2006; Courchesne and Pierce 2005; Welchew et al. 2005).

Neural systems in the brains of autistic patients are often inappropriately activated (Belmonte and Yurgelun-Todd 2003a; Brown 2005). In particular, abnormally enhanced sensory responses have been reported, and associated with this are deficits in orienting attention and transferring information to higher levels of processing (Townsend et al. 1996, 1999). According to Belmonte and Yurgelun-Todd (2003a, b), perceptual filtering selectivity in autism occurs in an all-or-none manner with little specificity for the task relevance of the stimulus. These authors suggest that perceptual filtering primarily depends on the control of general arousal rather than the

activation of a specific perceptual system. Since in many tasks requiring attention high-functioning persons with autism perform at close to normal levels (Belmonte 2000) despite generally high arousal and low selectivity, some compensatory mechanisms may be operating at a higher stage of processing to sort out the relevant stimuli from poorly discriminated background. One candidate mechanism has been suggested as the active inhibition of irrelevant distracters having passed through earlier filtering (Belmonte and Yurgelun-Todd 2003b).

There are several frontal ERP components that are considered as correlates of the cortical inhibitory processes in Go–NoGo-type task. In a three-stimuli oddball task, novel distracters are processed in a way similar to "NoGo" signal. The studies using visual NoGo-type tasks reported two effects in NoGo- vs. Go-ERPs: a negative wave at midline frontal sites with a latency of 150–400 ms, the "NoGo-N200" (or NoGo-N2), and a positive wave with maximum at the midline fronto-central area with a latency of 300–500 ms, the "NoGo-P300" (or NoGo-P3) (Falkenstein et al. 1999). The NoGo-N2 is thought to reflect a frontal inhibition mechanism which is active on NoGo trials (Roberts et al. 1994; Strik et al. 1998; Falkenstein et al. 2002; Bekker et al. 2004). The generators of the visual NoGo-N2 have been localized to inferior-lateral prefrontal cortex (Strik et al. 1998; Falkenstein et al. 2002). According to Falkenstein et al. (2002), NoGo-P3 could reflect a closure of a preceding inhibition process, whereas inhibition itself is reflected in the NoGo-N2. On the assumption that NoGo trials and zero delay stop signal are functionally equivalent, successful inhibition in stop-signal trials should exhibit the frontal N200 and P300 similar to the NoGo-N2 and NoGo-P3 potentials reflecting inhibition localized to the prefrontal cortex (Kok et al. 2004). Though most investigators agree that the NoGo-N2 and NoGo-P3 components are related to frontal inhibition seen during NoGo paradigms (reviewed by Falkenstein et al. 1999, 2002), others have shown that the anterior P300 in NoGo trials is not only due to response inhibition (Salisbury et al. 2004). In a task used in our visual oddball study (Sokhadze et al. 2010b), the frontal N200 was attenuated both to target and novels, while the frontal P300 was of the same amplitude both to targets and novels and was significantly prolonged. It is unlikely that frontal N200 and P300 were ERP correlates of the frontal inhibitory process of active suppression of motor response to novel targets in autism group.

The increased ratio of excitation/inhibition in key neural systems and high "cortical noise" has been considered core abnormalities in autism (Casanova et al. 2003; Rubenstein and Merzenich 2003). Schmitz et al. (2006) reported that although motor and higher cognitive inhibitory control appears to be preserved in high-functioning patients with ASD, there may be increased brain activation in task-relevant frontal and parietal cortices. This over-activation could be related to anatomical and neurodevelopmental abnormalities, or it could be caused by alternative, more effortful, cognitive strategies used by ASD subjects. There are numerous neuroimaging studies pointing to morphological asymmetry in autism, in particular greater volume and higher activation in the right rather than the left hemisphere (Courchesne et al. 2001; Herbert et al. 2005). Frontal and parietal ERP asymmetries in response to visual stimuli in individuals with autism are not sufficiently explored, and more experimental studies are needed in this direction. Our findings (Sokhadze et al. 2010b) of more profound ERP abnormalities at the right hemisphere during

novelty processing are in accord with neuroimaging and morphological studies demonstrating asymmetry of autistic brain (Herbert et al. 2005).

Contemporary models of neural connectivity outline the role of both integration and segregation in local and distal networks, their phase synchronization, and the large-scale integration of evoked and induced neural activity (Engel et al. 1991; Varela et al. 2001; Tallon-Baudry 2003; Tallon-Baudry et al. 2005). Our own neuropathological findings on minicolumnar abnormalities and a disrupted excitation/inhibition balance in autism (Casanova et al. 2002b, c, 2006a, b; Casanova 2005, 2006) along with other similar studies (e.g., Rubenstein and Merzenich 2003) provide additional support for the functional disconnectivity hypothesis in autism. Furthermore, we propose that excitation/inhibition abnormalities in autism could be indirectly assessed using ERP methods.

Patients with ASD compared to the typically developing control group in our study using two similar visual three-stimuli oddball tasks (Sokhadze et al. 2009a, b; Baruth et al. 2011) showed differences in reaction time and in response accuracy. At the anterior (frontal) topography, the ASD group showed significantly higher amplitudes and longer latencies of early components (e.g., P100, N100) and prolonged latencies of late ERP components (e.g., P2a, N200, P3a) to novel distracter stimuli at both hemispheres compared to controls; differences were more profound at the right hemisphere. Also, the ASD group showed increased latency of P3a to novels and reduced latency to targets in the right hemisphere compared to controls. In the left hemisphere, the ASD group showed increased latency to both target and novel stimuli compared to controls. These results indicate a reduced capacity for the ASD group to process distracters and orient to novelty, as longer latencies and higher amplitudes are indicative of more effortful processing of novel distracters in this group. ERP waveforms to target, standard, and novel stimuli were practically identical by latency and amplitude characteristics, which indicates very low selectivity for each stimulus category.

At the posterior (centro-parietal) topography, the ASD group in our study (Sokhadze et al. 2010b) showed significantly prolonged N100 latency and reduced amplitude of N2b to target stimuli when compared to controls, especially in the left hemisphere. Latency of the P3b component was prolonged to novel distracters more in this hemisphere, but there was no significant amplitude difference between groups to target and novel stimuli. In general, the autistic group showed increased latencies to novels but not to targets, especially in the right hemisphere. These results may indicate the overprocessing of information needed for the differentiation of target from nontarget novel stimuli in autism spectrum disorders.

The group of patients with autism showed delayed latencies of both early (N100) and late (P3b) posterior potentials to target stimuli, which is indicative of abnormalities of sustained attention compared to typical subjects (Baruth et al. 2010a, b; Sokhadze et al. 2010a, b). At the same time, the autism group exhibited a delayed frontal P3a to novel stimuli in both hemispheres, which can be considered a manifestation of impaired orientation to novelty, and decreased frontal associative and integrative function.

In general, abnormal activation in the parietal cortex during early processing of nontarget stimuli or novel distracters and at the same time under-activation of integrative regions in prefrontal cortices at the late stages of target processing

commonly occur in persons with autism in a visual target detection task employing rare novel distracters. This is reflected in a form of augmented and prolonged early frontal potentials and a delayed P3a component to novel stimuli, which would suggest low selectivity in preprocessing of sensory signal and at a later stage underactivation of integrative regions. However, our results did not indicate abnormality of the posterior P3b component amplitude to novel stimuli in autism compared to controls. This may indicate a reduction in the sensory discriminative ability of the ASD group. These results may be indicative of an over-connected network where sensory inputs evoke abnormally large summated potentials for unattended stimuli (standards, novel distracters) at all stages of stimulus processing with signs of a reduction in the selectivity of the activation.

Among the behavioral symptoms of autistic disorders, DSM-IV outlines that children with autism "[…] may insist on sameness and show resistance to or distress over trivial changes (e.g., a younger child may have a catastrophic reactions to a minor change in the environment such as a new set of curtains or a change in place at the dinner table)" (American Psychiatric Association 2000, p. 71). Other symptoms of autism relevant to cognitive ERP studies include "[…] odd responses to sensory stimuli (e.g., a high threshold for pain, oversensitivity to sounds or being touched, exaggerated reactions to light or odors, fascination with certain stimuli)" (American Psychiatric Association 2000, p. 72). Results of numerous studies (Courchesne et al. 1989; Ciesielski et al. 1990; Lincoln et al. 1993; Townsend et al. 2001; Ferri et al. 2003; Bomba and Pang 2004; Baruth et al. 2010a, b; Sokhadze et al. 2010a, b, 2012a, b) demonstrate that ERP measures may provide useful markers of over-reactivity to visual and auditory stimulation and, in particular, excessive reactivity and low habituation to novel distracters resulting from a low signal-to-noise ratio, deficient filtering selectivity, and impaired differentiation of task-relevant features in the incoming sensory stimuli. Therefore, behavioral and ERP findings could be directly linked to certain behavioral abnormalities and symptoms typical for ASD and may contribute to our understanding of autism.

Cognitive neuroscience studies of the brain mechanisms underlying the modulation of atypical behaviors characterizing autism are important in elucidating the neural mechanisms underlying autism. The examination of ERP measures during cognitive tasks with various sensory stimuli holds promising potential for contributing to our knowledge of autism. Enhanced understanding of the functional mechanisms of information-processing abnormalities based on ERP data could prove useful in diagnostic measurements of the level of cognitive impairments in autism spectrum disorders and in developing more effective interventions and social skill training programs focused on autistic children's behavioral and mental health.

4.4 Error-Related Potentials in Autism

Application of ERP methodology is not limited only to evaluation of responses to sensory stimuli, but they also can be used to assess motor response-related processes. Some ERPs can be used to understand response-related neural processes.

There is growing evidence that executive function deficits may contribute to these core symptoms (Hill 2004) and executive abnormalities remain on a major autistic symptoms list. One important executive function known to be compromised in ASD is the ability to select a contextually appropriate response among several competing ones and simultaneously inhibit contextually inappropriate responses to avoid committing an error. Another executive deficit observed during performance on speeded reaction time tasks in autism is manifested in an abnormality related to response error monitoring and post-error response correction.

Current theory and research suggests that deficits in response monitoring may contribute to social–emotional and social–cognitive impairments in autism (Henderson et al. 2006; Sokhadze et al. 2010a, b, 2012a, b). Executive deficit hypotheses of autism emphasize that many of the everyday behaviors of autistic individuals, such as perseverative responding, repetitive behaviors, poor imitation skills, and joint attention impairments, may involve an inability to consistently and accurately monitor ongoing behaviors (Mundy 2003). Therefore, impairments specific to self-monitoring function have been already outlined in earlier models of autism (Russell 1997; Russell and Jarrold 1998). Several recent reports (Henderson et al. 2006; Bogte et al. 2007; Franken et al. 2007; Thakkar et al. 2008; Vlamings et al. 2008) indicate that children and adult patients with ASD show reduced error processing and deficient behavioral correction after an error is committed. This finding could be explained as a reflection of ASD patients' lower sensitivity to behavioral errors and/or a reduced behavior correction ability.

Performance on behavioral tasks is monitored by a brain system that is responsive to errors (Gehring et al. 1993; Falkenstein et al. 2000; Gehring and Knight 2000; Luu et al. 2000, 2003). Evidence from functional magnetic resonance imaging (fMRI), quantitative EEG, and ERP studies outlines that error monitoring is a function of the medial frontal cortex (MFC), including the supplementary eye fields, rostral cingulate motor area, and dorsal anterior cingulate cortex (ACC) (Ridderinkhof et al. 2004). Our neuropathological studies in autism suggest the presence of significant minicolumnar abnormalities in brain regions related to error monitoring, i.e., MFC and ACC (for references see Casanova et al. 2006a, b).

Error sensitivity can be readily examined by measuring response-locked event-related potential components associated with brain responses to errors. Two specific components relevant in this context are the error-related negativity (ERN, more rarely referred to as Ne) and the error-related positivity (Pe). The ERN is a response-locked negative ERP deflection, emerging between 0 ms and 150 ms after the onset of the incorrect behavioral response—a commission error. Usually this ERN is followed by a positive wave referred to as the Pe potential. Although there is discussion about the exact meaning of the Pe (Overbeek et al. 2005), most studies indicate that the Pe is related to the conscious recognition of the error (Nieuwenhuis et al. 2001) or the attribution of motivational significance to the committed error (Falkenstein et al. 2000). This suggests that the ERN reflects an initial automatic brain response as a result of an error, and the Pe possibly indicates the conscious reflection and comprehension of the error (Overbeek et al. 2005). The magnitude of the ERN is associated with behavioral evidence of self-monitoring (i.e., self-correction and post-error slowing responses) and therefore is interpreted as a

biomarker of error processing (van Veen and Carter 2002). Dipole modeling has localized ERN sources to the caudal ACC, while Pe has been localized to the more rostral ACC division (Bush et al. 2000; Gehring and Knight 2000; van Veen and Carter 2002; West 2003; Herrmann et al. 2004). ERN and Pe are generally accepted as neural indices of response-monitoring processes in psychophysiological research and clinical neurophysiology.

One of the important research questions is whether this error-related frontal activity is associated with a premorbid trait reflecting an initial deficiency of behavioral control and regulation and whether this deficit can be generated as a result of neuropathological states associated with behavioral control deficits (e.g., pervasive developmental disorders). Several clinical research studies have demonstrated an excessive error processing in patients with obsessive–compulsive disorders (OCD) (Johannes et al. 2001), anxiety disorders (Markela-Lerenc et al. 2004), and Tourette syndrome (Gehring et al. 2000). On the contrary, reduced error-processing manifestations were reported in borderline personality disorder (de Bruijn et al. 2006) and schizophrenia (Mathalon et al. 2002). In psychiatric studies, a decreased ERN is typically related to increased severity of psychomotor poverty symptoms (Bates et al. 2004). Furthermore, error processing has also been found to be reduced in nonclinical traits such as high impulsivity (Ruchsow et al. 2005).

Neuroanatomically and functionally, the anterior cingulate cortex (ACC) provides an interface between frontal action selection processes, limbic emotion or motivation processes, and motor output regulation (Coles et al. 2001; Holroyd and Coles 2002; Taylor et al. 2007). The integral role of the ACC in self-monitoring and guiding attention in goal-directed actions suggests that it may be an important focus for autism research. Disturbances in attention regulation and behavioral rigidity may result in social-orienting deficits and a chronic disruption of social information processing and social learning that together may contribute to the social–cognitive and emotional deficits observed in autistic children (Mundy 1995; Dawson et al. 1998; Mundy and Neal 2000; Klin et al. 2003).

Several neuroimaging studies (Hall et al. 2003; Barnea-Goraly et al. 2004) suggest that anomalous functioning of the ACC may distinguish between individuals with autism and controls. Haznedar et al. (2000) observed that a sample of children with autism displayed hypometabolism in the right ACC relative to controls, while an Asperger disorder subsample displayed left ACC hypometabolism relative to controls. There have been also several ERN-based empirical demonstrations of connections between ACC function and autism. Children with high-functioning autism displayed longer ERN latencies but did not differ in amplitude of the ERN relative to children in the control group in the Eriksen flanker task (Henderson et al. 2006). There is other evidence of abnormal response monitoring in autism, in particular reduced error self-correction (Russell and Jarrold 1998) and reduced post-error slowing, a compensatory mechanism to improve performance on the subsequent trial (Bogte et al. 2007). Since the evaluation of ongoing behavior and its consequences is necessary to determine whether or not current behavior adjustment strategies should be maintained, abnormal response monitoring and deficient adaptive correction may contribute to the behavioral inflexibility and stereotypy associated with ASD.

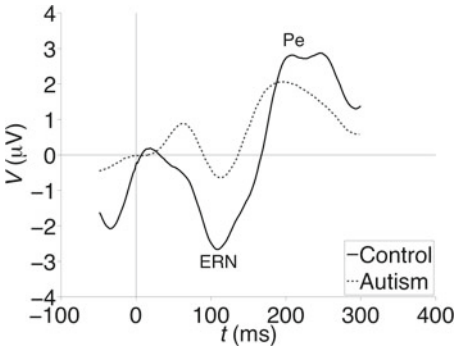

Fig. 4.3 Error-related negativity (ERN) and positivity (Pe) from the frontal midline EEG site in two groups (controls, ASD). Grand average waveforms ($N = 16$ per group) show more negative amplitude and shorter latency of the ERN in control group as compared to the ASD group

In our recent studies on error monitoring in autism (Sokhadze et al. 2010a, b, 2012a, b), we showed that the ERN and the Pe component of the response-locked ERP were substantially decreased in children with autism as compared to typically developing (TD) controls and even children with ADHD. In particular the amplitude of ERN was less negative, and latency of both ERN and Pe was prolonged in the ASD group as compared to the TD children (Fig. 4.3). The ERN is an electroencephalographic measure associated with the commission of errors, thought to be independent of conscious perception (Franken et al. 2007), while the Pe is thought to reflect the motivational or emotional significance of the error or, in other words, the conscious evaluation of the error (Overbeek et al. 2005). The findings that both ERN and Pe are altered in autism may suggest that ASD patients are not only less sensitive to committed errors but that they are also less aware of their errors probably attributing less significance to them. Inadequate and inflexible responsiveness to errors may underlie one of the typical characteristics of autism spectrum disorders, namely, the persistence of stereotyped repetitive behaviors.

It cannot be ruled out that ERN and Pe impairments are influenced by deficits in earlier perceptual processes, or attentional and working memory processes in children with autism, that might be reflected in altered stimulus-locked early and late ERPs. Though we did not observe a significant effect of group on the frontal N200 amplitude (Sokhadze et al. 2009a, b), we found a significantly delayed latency of the N200 to novel distracters in a similar three-category oddball task suggesting that early processes taking place before the response may also be affected in autism. It has been suggested (Yeung et al. 2004; Yeung and Cohen 2006) that both the response-locked ERN and the stimulus-locked frontal N200 might reflect similar processes (i.e., response conflict detection and monitoring) and have similar neural correlates (i.e., the ACC).

On the behavioral level, we found no group differences in RT and only modest group differences between the percentages of commission (and not omission) error in the visual novelty oddball and illusory figure tasks (Sokhadze et al. 2010a, 2012b). After an error, ASD patients did not show accuracy improvement through post-error RT slowing as typical controls did (Fig. 4.4). Normally, performance on these trials is improved as result of a change in speed–accuracy strategy which

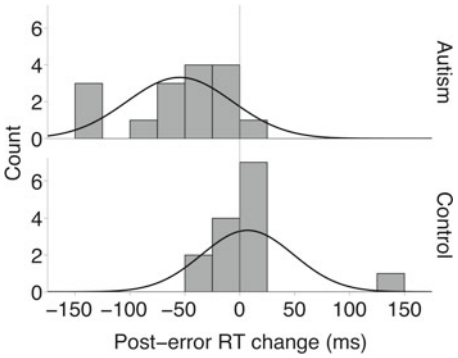

Fig. 4.4 Histogram of distribution of individual post-error reaction time (RT) in children with autism and typically developing controls ($N = 16$/group). Control group demonstrates slower (*positive*) post-error RTs compared to correct response RTs. The ASD group shows speeding of post-error RTs with a *negative* peak of distribution curve

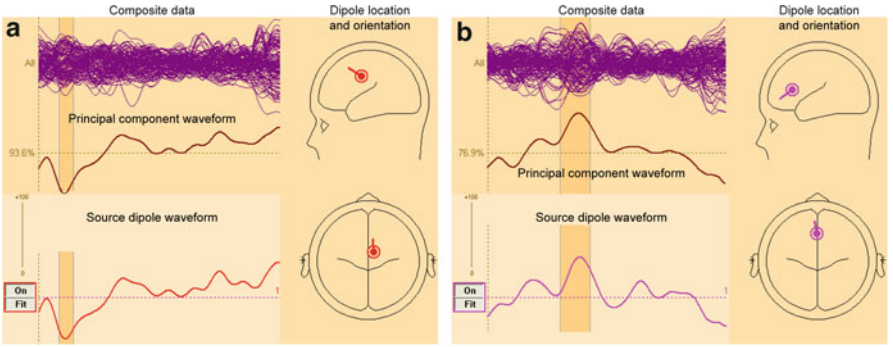

Fig. 4.5 (**a**) Dipole source localization and orientation created in BESA show a single dipole with 93.6 % loading (using Principal Component Analysis [PCA]) placed in the caudal division of the ACC for the ERN (grand average for the control group, $N = 14$). (**b**) Dipole source localization and orientation created in BESA show a single dipole with 76.9 % loading (using PCA) placed in the rostral division of the ACC for the Pe (grand average for the control group, *004E* = 14)

reflects executive control functioning (Burle et al. 2002). The worsened post-error performance of ASD children suggests the presence of an executive control deficiency. The impairment of adaptive error-correction behavior may have important consequences in daily life as optimal error correction is necessary for adequate behavioral responses.

As demonstrated in previous studies (Ridderinkhof et al. 2004), the posterior medial frontal cortex, more specifically the rostral ACC division, is the main brain area responsible for error processing, suggesting that ASD patients have reduced posterior medial frontal cortex functioning. This area is involved when there is a need for adjustments to achieve goals (Ridderinkhof et al. 2004). Figure 4.5 shows dipole source localization for ERN and Pe.

The findings pointing that children with ASD have an impaired ability to improve their response accuracy by slowing down the response speed on post-error trials correspond with this notion. However, it is necessary to take into account that observed significant group differences between ASD and typical controls are

manifested not only in the behavioral performance measures on reaction time tasks (RT, error rate) and associated response-monitoring indices (both to erroneous and correct) but also in terms of amplitude and latency characteristics of early- and middle-latency ERP components preceding motor response selection (frontal and parietal P100, N100, P200, N200) and those reflecting context update and closure (e.g., P300, N450) in visual oddball task (Sokhadze et al. 2009b) and various auditory tasks (Bomba and Pang 2004). The sum of the group differences across these behavioral stimulus- and response-averaged ERP indices of ASD patients' performance reflects global deficits in attentional processes, and more specifically deficits in effective differentiation of target and distracter stimuli. This latter interpretation is supported by the significant differences between the ASD patients and typically developing controls in terms of the stimulus-locked ERP amplitudes and latencies, and the correlation between subjects' behavioral performance measures and specific ERP components magnitude.

Post-error adaptive correction of responses might be explained by some recent neurobiological findings. There are reports about an excessive preservation of short-distance connections (i.e., local over-connectivity) and relatively poor long-distance connections (i.e., distant under-connectivity) in the neocortex of individuals with autism (Just et al. 2004; Casanova 2005, 2006; Williams and Casanova 2010). These cortical connectivity abnormalities may explain why persons with autism tend to focus on details rather than perceiving the whole Gestalt. This overfocusing on details may imply an excessively laborious and ineffective way of handling each trial in the cognitive test and lower availability of resources after an error when effort is needed to react appropriately. This may result in insufficient activation of the ACC (Bogte et al. 2007), and thus error detection and post-error reaction may be hampered (Bauman and Kemper 2005; Minshew et al. 2005). Structural and functional deficiencies of the ACC may contribute to the atypical development of joint attention and social cognition in autism (Mundy 2003). Such interpretation of the results of the ERN/Pe deficits found in several studies (Henderson et al. 2006; Bogte et al. 2007; Sokhadze et al. 2010a) is consistent with many aspects of theory and research that suggests that ACC-mediated response monitoring may contribute to social–emotional and social–cognitive development in autism (Mundy 2003). However, while emphasizing the possible role of ACC-related self-monitoring deficits in autism, Mundy (2003) also noted that according to Devinsky and Luciano (1993), these ACC impairment-related behavioral deficits emerge only when they are combined with disturbances in other related functional neural networks, e.g., dorsolateral prefrontal cortex.

4.5 Applications of ERP in Autism Research

There are several important practical applications of ERP testing in autism. The first one is the application of ERP tests for functional evaluation as this method has substantial diagnostic potential. The question of using ERP parameters as a diagnostic tool was discussed by Kemner et al. (1999), who used multivariate analysis and found

that several parameters (mainly P300) showed differences among patients with autism, attention deficit hyperactivity disorder (ADHD), multiple complex developmental disorder (MCDD), and dyslexia. When ERP parameters were used as variables in discriminate analysis, it was possible to classify several child psychiatric groups and a normal control group well above chance level, with classification occurring in 46 % of the cases. When only clinical groups were compared (ASD, ADHD, MCDD, dyslexia), the classification correctness reached 60 % (Kemner et al. 1999). However, autism is only one of numerous psychiatric and neurological disorders in which parietal P300 (P3b) is abnormal. Attenuated P3b was found in schizophrenia, bipolar disorder, ADHD, and alcoholism to name a few (Pritchard 1986; Picton 1992; Polich and Herbst 2000) and cannot be considered as a specific marker for ASD. Expanding the topographical areas of ERP measurements (e.g., frontal, parietal, etc.) and adding earlier potentials (e.g., N100) and error-related potentials (i.e., ERN and Pe) may increase the diagnostic potential for clinical and functional evaluations of ASD.

Our error-related potential findings (Sokhadze et al. 2010a, 2012a, b) reveal that autism is associated with reduced error processing and impaired behavioral correction after an error is made. Because adequate error processing is necessary for optimal behavioral performance, it is plausible that these deficits contribute to the maintenance of the preservative behaviors typical for autism. Abnormal response monitoring and correction functions observed in behavioral and electrocortical indices of the ACC in ASD that might be related to the restricted, repetitive behavior typical of this neurodevelopmental disorder. This abnormal function may result from compromised functional and structural connectivity in the neural circuitry subserving response monitoring and error correction. These findings suggest that functional abnormalities of the ACC reflected in lower amplitude and delayed ERN and Pe measures may compromise response monitoring and contribute to repetition of erroneous behaviors in ASD. Impairments in an ability to correctly and timely evaluate committed error and to learn from errors may lead to behavior that is rigid and repetitive rather than adaptively guided by action outcomes. Deficits in adjustments of erratic behavior during interaction with peers may as well affect social interaction of children with autism. Elucidating the neurobiological basis and clinical significance of response monitoring and correction deficits in ASD represents a promising direction for further quantitative EEG-based research. The ERN and Pe along with behavioral performance measures can be used as functional outcome measures to assess the effectiveness of behavioral interventions (e.g., social skills training) or neurotherapies (e.g., repetitive transcranial magnetic stimulation [rTMS] or neurofeedback) in children with autism spectrum disorders and thus may have important practical implications. In a recent study (Sokhadze et al. 2012a), we showed significant improvement in ERN and behavioral measures (error rate, post-error RT slowing) in children with autism following 12 sessions of low-frequency rTMS.

The application of ERP indices in a standardized visual or auditory oddball tasks as an outcome measure in ASD evaluation seems to be a feasible approach considering the growing interest in quantitative electroencephalographic (qEEG) assessments of children with autism (Coben, presented at the 16th ISNR Annual Conference, San Antonio, August 26–31, 2008) and the recent presentations, pilot publications,

and reviews on EEG biofeedback applications in ASD (presented by Linden, by Pineda, and by Coben, each at the 15th ISNR Annual Conference, San Diego, September 6–9, 2007). Furthermore, other pilot neurofeedback studies in autism offer auxiliary support (Sichel et al. 1995; Jarusiewicz 2002; Scolnick 2005; Coben and Padolsky 2007).

In another study underway, we examined the effects of low-frequency, repetitive TMS on behavior and social functioning in persons with autism along with EEG and ERP outcomes (Casanova et al. 2012). We proposed that post-TMS changes in the treatment group can be detected during repeated cognitive tests using the same functional outcome measures (e.g., P3a and P3b ERP components). Our hypothesis in this study was that low-frequency rTMS of the dorsolateral prefrontal cortex will result in an alteration of cortical inhibition through the activation of inhibitory GABAergic interneurons leading to an improvement in the excitatory/inhibitory balance. The instruments for social and behavioral functioning evaluation (Guy 1976; Aman and Singh 1994; Bodfish et al. 2000; Constantino and Gruber 2005) were selected with the hypothesis that rTMS sessions will result in reduced irritability and hyperactivity, reduced obsessive–compulsive and stereotyped behavior, and improved social awareness. For the functional outcome measures, we selected a novelty test to assess changes in electrocortical measures, such as amplitude and latency of early and late frontal and parietal ERPs. We believe that ERP evaluation in a repeated posttreatment oddball test can serve as valuable outcome measurement for autistic patients. The results of the ERP studies help understand the specific behavioral, social communication, and neurocognitive deficits associated with developmental abnormalities of functions within cortical circuitry and thereby contribute to better understanding the brain substrates of attentional and cognitive processing dysfunctions typical for autism spectrum disorders.

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Biography

Estate M. Sokhadze received a Ph.D. in human physiology in 1988 (Novosibirsk, Russia). He completed a postdoctoral fellowship in psychopharmacology at Wake Forest University in 2001–2003 and postdoctoral training in cognitive neuroscience at Rice University in 2004. Currently, Dr. Sokhadze is an associate professor of Psychiatry and Behavioral Sciences at the University of Louisville and is a director of the Evoked Potential Lab at Cognitive Neuroscience Labs. His research interests include application of dense-array EEG/ERP brain mapping, neurofeedback, TMS, and other applied psychophysiological techniques in psychiatric research. Specific psychopathology areas of interest are substance abuse, PTSD, autism, ADHD, conversion disorder, bipolar disorder, and comorbid mental conditions. He has more than 25 years of experience in applied psychophysiology and clinical neurosciences.

Joshua Baruth graduated from the Department of Anatomical Sciences and Neurobiology at the University of Louisville School of Medicine. Joshua's research has focused primarily on the treatment of autism spectrum disorders with transcranial magnetic stimulation. He received his BA in classical languages and pre-medicine from the University of Kansas in 2005 and his master's degree in anatomical sciences and neurobiology from the University of Louisville in 2009 and his Ph.D. in 2010. He is currently at Mayo Clinic in Minnesota as a postdoc fellow.

Allan Tasman, professor and chairman of the Department of Psychiatry and Behavioral Sciences at the University of Louisville since 1991, completed undergraduate work at Franklin & Marshall College, medical school at the University of Kentucky, and psychiatric residency at the University of Cincinnati where he was chief resident. He also is a graduate of the Western New England Psychoanalytic Institute. In the American Psychiatric Association, he served as scientific program chairman, vice president, and president. In 2005, he was elected to a 6-year term as secretary for education of the World Psychiatric Association. He has authored or edited 32 psychiatric textbooks and monographs, over 200 peer-reviewed publications, chapters, and abstracts, and numerous national and international presentations. He is senior editor of the first, second, and third editions of a comprehensive textbook, *Psychiatry*.

Manuel F. Casanova is a board-certified neurologist trained in clinical electroencephalography and evoked response potentials. His research focus is autism spectrum disorders. Dr. Casanova is an endowed chair professor and is the associate chair for research in the Department of Psychiatry and Behavioral Sciences at the University of Louisville. He has over 20 years of experience in the neurosciences. During the last 5 years, he has published 43 refereed articles, edited 3 books, wrote 4 letters to the editor, and has completed 74 congressional presentations worldwide. He is one of the founders of the Autism Center at the University of Louisville. He was principal investigator on several federal grants, and now he is a PI on an NIH Eureka grant aimed at the application of TMS in autism.

Chapter 5
Evoked and Induced Gamma-Frequency Oscillations in Autism

Manuel F. Casanova, Joshua Baruth, Ayman S. El-Baz, Guela E. Sokhadze, Marie Hensley, and Estate M. Sokhadze

5.1 Minicolumnar Neuropathology Model of Autism and EEG Gamma

Recent studies by our group have characterized the neuropathology of autism as that of a minicolumnopathy. Postmortem studies using computerized image analysis of pyramidal cell arrays have found that the brains of autistic individuals have smaller minicolumns with most of the decrease stemming from a reduction in its peripheral

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neuropil space, with little, if any reduction, in their core space. This finding has been reproduced using different techniques (e.g., GLI) and independent populations (Casanova et al. 2002a, b, c, 2006a, b). It is now known that minicolumnar width reduction in autism spans supragranular, granular, and infragranular layers (Casanova et al. 2010). The most parsimonious explanation for the findings is the possible abnormality of an anatomical element in common to all layers. The peripheral neuropil space of minicolumns provides, among other things, for inhibitory elements distributed throughout all of its laminae. This is the so-called shower curtain of inhibition of the minicolumn described by Szentágothai and Arbib (1975). Our findings therefore suggest a deficit within the inhibitory elements that surround the cell minicolumn (Casanova et al. 2006a).

The anatomical disposition of inhibitory elements within the shower curtain of inhibition provides clues as to their function. While tangentially arrayed basket cells function, in part, to coordinate activity among remote neuronal ensembles, by contrast, radially oriented inhibitory interneurons prominently located in the peripheral neuropil space surrounding pyramidal cell columns likely function to segregate columns from interference, both from other minicolumns within an array and from fields of activity or inhibition in neighboring minicolumnar arrays (Casanova et al. 2003). The finding suggests a mechanistic explanation to the inhibitory/excitatory imbalance in autism and a possible explanation to the multifocal seizures often observed in this condition (Casanova et al. 2003).

Oscillations of pyramidal cells in minicolumns and across assemblies of minicolumns are maintained by networks of different species of inhibitory, GABA-expressing interneurons. In this regard interneurons make a critical contribution to the generation of network oscillations and help synchronize the activity of pyramidal cells during transient brain states (Mann and Paulsen 2007). Local excitatory–inhibitory interactions help shape neuronal representations of sensory, motor, and cognitive variables and produce local gamma-band oscillations in 30–80 Hz range (Donner and Siegel 2011). The excitatory–inhibitory bias caused by faulty pyramidal cell-interneuronal dyads provides a receptive scenario to gamma-frequency abnormalities in autism.

Gamma frequencies are closely associated with sensory processing, working memory, attention, and many other cognitive domains (Ward 2003; Jensen et al. 2007). The brain's limited long-range wiring cannot directly sustain coordinated activity across arbitrary cortical locations, but it can convey patterns of synchronous activity as oscillatory neuronal fluxes, represented by local field potentials measured by EEG. Coordination of oscillations at varying interacting frequencies allows for relatively efficient and unconstrained segregation in varying forms and across hierarchical cortical levels. Disrupted patterns of coordinated oscillatory output in distributed minicolumnar networks might be associated with cortical "disconnection" in autism. More specifically, altered oscillatory activity in developing cortical circuits may contribute to impaired development of intra-areal and transcortical connections giving rise to a bias in short (e.g., arcuate) vs. long corticocortical projections (e.g., commissural fibers) (Casanova et al. 2006a, b, 2009). The pervasive nature of abnormalities ingrained in this oscillatory activity bears significant

analogy to the cognitive deficits observed in autism. It is therefore unsurprising that gamma oscillations have been claimed to be directly related to the pathophysiology of autism (Sohal 2012). To the authors' knowledge every study on gamma frequencies in autism has been abnormal.

5.2 Functional Significance of Gamma Oscillations

Electroencephalography (EEG) has been used to decompose oscillatory patterns into several frequency bands: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (30–80 Hz), each of which operates over various spatiotemporal scales to control cortical activity. High-frequency gamma-band oscillations are most directly associated with entrainment of local networks. Strong evidence indicates that this gamma-frequency activity is associated with binding of perceptual features in animals (Herrmann and Knight 2001). Human experiments have also found that induced gamma activity correlates with binding (Kaiser 2003). Binding of widely distributed cell assemblies by synchronization of their gamma-frequency activity is thought to underlie cohesive stimulus representation in the human brain (Keil et al. 1999; Rodriguez et al. 1999; von Stein et al. 1999; Bertrand and Tallon-Baudry 2000; Kahana 2006; Pavlova et al. 2006). Increased gamma activity has been most widely associated with top-down attentional processing and object perception (Rodriguez et al. 1999; Gruber et al. 2001; Fell et al. 2003; Nakatani et al. 2005) subserving Gestalt pattern perception (von Stein et al. 1999; Herrmann and Mecklinger 2000).

Contemporary models of neural connectivity outline the role of integration and segregation of both local and distal networks, their phase synchronization and large-scale integration of evoked and induced neural activity (Tallon-Baudry et al. 1998, 2005; Varela et al. 2001; Tallon-Baudry 2003). Functional coupling and decoupling of neural assemblies could be analyzed within specific time and frequency windows of electrocortical activity. Gamma-band activity can be divided into either evoked or induced: evoked gamma-band activity has been identified at a latency of around 100 ms after stimulus onset (Bertrand and Tallon-Baudry 2000; Herrmann and Mecklinger 2000) and is phase-locked to the onset of the stimulus; induced gamma-band activity occurs later with a variable onset although it has been reported to start at around 250 ms (Brown et al. 2005) (Fig. 5.1). It has been proposed that evoked gamma-band activity reflects the early sensory processing and the binding of perceptual information within the same cortical area (i.e., intra-areal), whereas induced gamma-band activity reflects the binding of feed-forward and feedback processing in a whole network of cortical areas (corticocortical) (Shibata et al. 1999; Müller et al. 2000; Brown et al. 2005). Variations of such activity have been termed event-related synchronization and desynchronization (ERS/ERD) (Pfurtscheller and Aranibar 1977) or event-related spectral perturbations (ERSP) (Makeig et al. 2004) and have been associated with the activation of task-relevant neuronal assemblies (Pfurtscheller and Lopes da Silva 1999; Rippon et al. 2007).

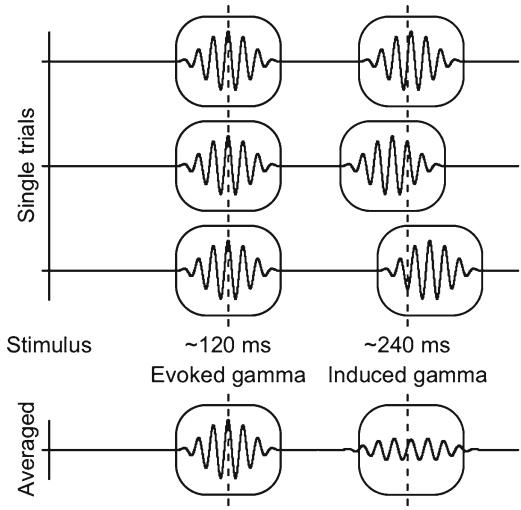

Fig. 5.1 Schematic representation of early evoked (peaking around 120 ms poststimulus) and late induced gamma (peaking around 240 ms) oscillations in single trials and averaged gamma response during visual oddball task

5.3 Abnormalities of Gamma Activity in Autism

Excitatory output of projection neurons is modulated and coordinated by oscillatory electrocortical activity of area-specific arrays of inhibitory interneurons. Phasic synchronization of these local oscillation patterns may provide a basis for functional integration across widely distributed cortical networks (Müller et al. 2000; Varela et al. 2001; Tallon-Baudry 2003, 2005). Visual and auditory perception anomalies, as well as some features of language processing and social communication deficits, and executive dysfunctions associated with "weak central coherence" in autism (Frith and Happé 1994; Morgan et al. 2003; Mottron et al. 2003; Plaisted et al. 2003; Happé and Frith 2006) may be attributed to reduced gamma-frequency synchronization and decreased temporal binding of activity between networks processing local features.

Disrupted visual perceptual congruence in individuals with autism is illustrated by a study (Brown 2005) in which subjects were presented with a visual-shape illusion (Kanizsa 1976). The autistic individuals exhibited a burst of gamma activity in posterior areas at 300 ms, which was greater in power and duration than the corresponding gamma response in controls. In another study Brown et al. (2005) could not find reaction time or accuracy differences between groups in a task of Kanizsa figure identification, but they showed significant task-related differences in gamma activity. Control participants showed typical gamma-band activity over parietal regions at around 350 ms, while autistic participants showed overall increased activity, including an early 100 ms gamma peak and a late induced peak, occurring earlier than that shown by the control group. The authors interpreted the abnormal gamma activity to reflect decreased "signal to noise" due to decreased inhibitory processing. Brock et al. (2002) described the parallels between the psychological

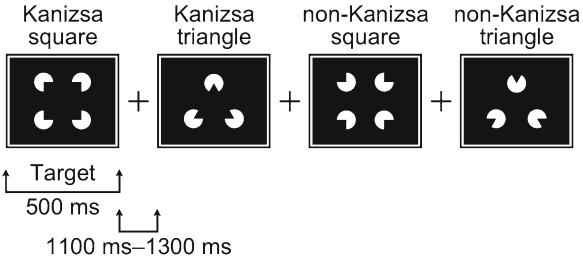

Fig. 5.2 Kanizsa and non-Kanizsa figures were used as stimulus materials in this experiment. In particular, the stimulus types used in the experiment are Kanizsa *square* (target), Kanizsa *triangle*, non-Kanizsa *square*, and non-Kanizsa *triangle*. The nontarget Kanizsa *triangle* was introduced for the differentiation of processing Kanizsa figures and targets. The stimuli consisted of either three or four inducer disks which are considered the shape feature and either do or do not constitute an illusory figure (*square*, *triangle*). Kanizsa illusory figures readily induce gamma response during perceptual processing (Herrmann and Mecklinger 2001; Brown 2005)

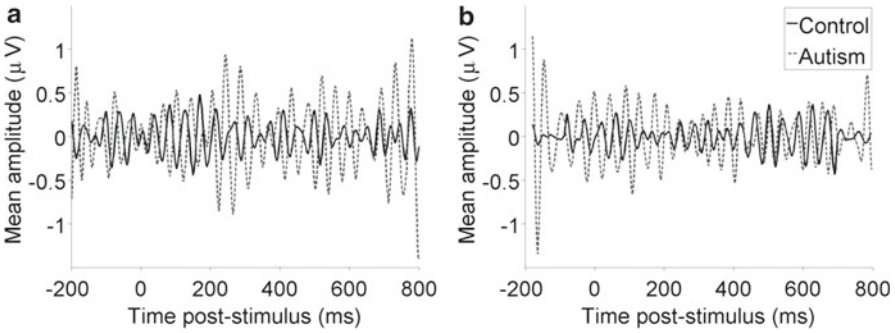

Fig. 5.3 Gamma-frequency oscillations in response to Kanizsa target (**a**) and nontarget (**b**) stimuli. Children with autism show higher power of late (240–400 ms poststimulus) gamma oscillations in response to the target Kanizsa stimulus and higher power of early (40–180 ms poststimulus) gamma oscillations in response to the nontarget Kanizsa stimulus

model of "central coherence" in information processing (Frith and Happé 1994) and the neuroscience model of neural integration or "temporal binding." They proposed that autism is associated with abnormalities of information integration that is caused by a reduction in the connectivity between specialized local neural networks in the brain and possible over-connectivity within the isolated individual neural assemblies. This concept was further elaborated in an "impaired connectivity" hypothesis of autism (Rippon et al. 2007), which summarized theoretical and empirical advances in research implicating disordered connectivity in autism. The authors highlighted recent developments in the analysis of the temporal binding of information and the relevance of gamma activity to current models of structural and effective connectivity based on the balance between excitatory and inhibitory cortical activity (Casanova et al. 2002a, b, c; Rubenstein and Merzenich 2003; Belmonte et al. 2004a, b) (Figs. 5.2 and 5.3).

It has been proposed that "weak central coherence" (Frith and Happé 1994; Morgan et al. 2003; Mottron et al. 2003; Plaisted et al. 2003; Happé and Frith 2006; Murias et al. 2007) in autism could result from a reduction in the integration of specialized local networks in the brain caused by a deficit in temporal binding (Brock et al. 2002; Rippon et al. 2007). Audiovisual perception anomalies associated with weak central coherence may be attributed to a reduction in synchronization of gamma activity between networks processing local features and can explain some of the features of language deficits, executive dysfunctions, and other impairments in social communication in autism. Excessive but not synchronized gamma can be linked to a reduction in the ability to focus attention. In autism, uninhibited gamma activity suggests that none of the circuits in the brain can come to dominance because too many of them are active simultaneously (Brown et al. 2005). A proposed "temporal binding deficit" hypothesis of autism (Grice et al. 2001; Brock et al. 2002; Rippon et al. 2007) suggests that many features of autism, such as superiority in processing of detail (local processing) and disadvantage in global processing, necessitating integration of information either over space, time, or context, can be explained by a failure of binding between cortical areas. Abnormal gamma activation would suggest disrupted neural signaling and would support the hypothesis of abnormal regional activation patterns.

5.4 Investigation of Evoked and Induced Gamma Responses in Autism

It is well known that networks of inhibitory interneurons acting as GABA-gated pacemakers are critically involved in gamma oscillations (Grothe and Klump 2000; Whittington et al. 2000). Electrophysiological research has provided evidence that gamma activity is a physiological indicator of the co-activation of cortical cells engaged in processing visual stimuli (Singer and Gray 1995; Tallon-Baudry and Bertrand 1999; Keil et al. 2001) and integrating different features of a stimulus (Müller et al. 2000). The onset of a visual stimulus gives rise to a burst of gamma activity over occipital sites, and when more complex tasks are undertaken, discrete bursts of gamma activity have been identified overlying cortical regions thought to be engaged in those tasks (Brown et al. 2005). For example, tasks involving attention modulation or the top-down integration of features give rise to simultaneous bursts of gamma over frontal and occipitoparietal regions (Rodriguez et al. 1999; Müller et al. 2000; Müller and Gruber 2001).

Kanizsa illusory figures (Kanizsa 1976) have been shown to produce gamma oscillation bursts during visual cognitive tasks (Tallon-Baudry et al. 1996; Herrmann et al. 1999). Kanizsa stimuli consist of inducer disks of a shape feature and either constitute an illusory figure (square, triangle) or not (colinearity feature); in non-impaired individuals, gamma activity has been shown to increase during "target-present" compared to "target-absent" trials (Müller et al. 1996; Tallon-Baudry et al. 1996; Brown et al. 2005). In several studies, Kanizsa figures were employed as stimuli in an oddball task paradigm to investigate effects of target classification and

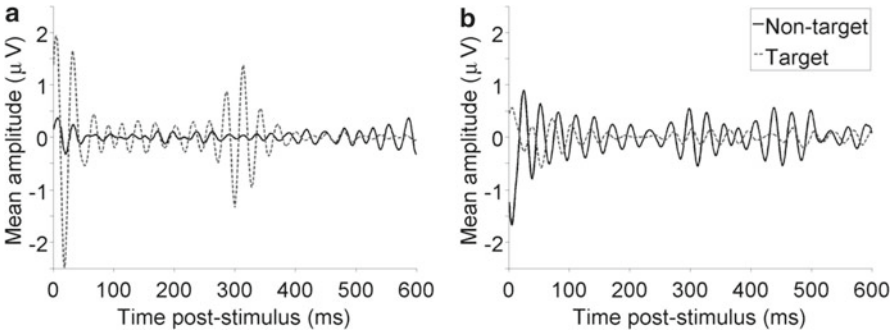

Fig. 5.4 Frontal (F1) induced gamma responses (peak of oscillations close to 300 ms) in children with autism spectrum disorder (ASD, $N = 15$) and age-matched controls ($N = 15$) in Kanizsa oddball task. The control group (**a**) shows higher amplitude of gamma burst to target stimuli, while the ASD group (**b**) shows higher gamma response to nontarget Kanizsa figures

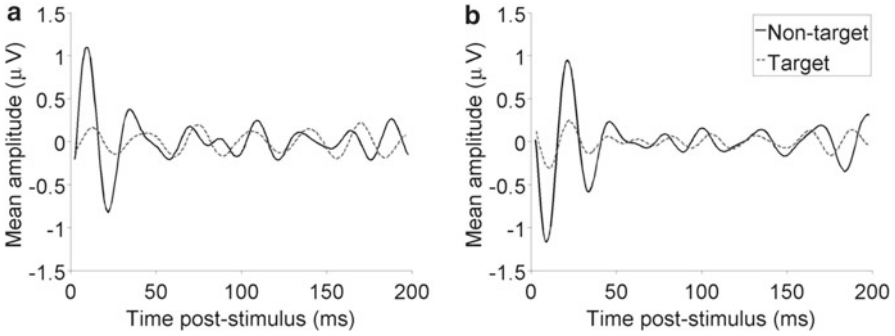

Fig. 5.5 Early evoked gamma oscillations to target and nontarget rare Kanizsa figures at the *left* lateral frontal site F7 (**a**) and parietal site P7 (**b**) in a group of children with autism. Evoked gamma to nontarget stimuli is comparable and even larger in amplitude than gamma response to target stimuli

discrimination between illusory stimulus features (Tallon-Baudry et al. 1998; Herrmann and Mecklinger 2000; Böttger et al. 2002; Brown 2005; Sokhadze et al. 2009a). In our study of gamma activity in autism (Sokhadze et al. 2009a; Baruth et al. 2010; Casanova et al. 2012), we used a modification of such oddball test where subjects performed a visual discrimination task which required a response to target Kanizsa squares among nontarget Kanizsa triangles and non-Kanizsa figures. This task was used to examine gamma-band EEG activity and event-related potentials (ERP). Power of induced gamma oscillations (at twelve left and right frontal, central, parietal, and occipital EEG sites, 30–80 Hz range, in μV^2) was analyzed using wavelet transformation. Density of induced power of gamma oscillations ($\mu V^2/Hz$) and power density difference between gamma response to nontarget and target Kanizsa stimuli (target minus nontarget Kanizsa conditions) were also calculated and analyzed. Power of gamma oscillations in response to nontarget Kanizsa and non-Kanizsa standard stimuli was higher in autism group at the left frontal (F1, F7), left and right parietal (P1, P2, P7, P8), and occipital (O1, O2) EEG channels (Figs. 5.4 and 5.5).

Group (control, autism) differences in gamma oscillation power to nontarget and target Kanizsa stimuli were better expressed over the lateral frontal (F7, F8) and parietal (P7, P8) EEG sites. A *Stimulus* (target, nontarget) × *Group* (autism, control) interaction was highly significant for all recording sites ($p < 0.001$) and described as higher gamma power to nontargets in autism group compared to controls. We found also a *Hemisphere* × *Group* interaction across the lateral frontal and parietal sites, with difference between target and nontarget stimuli being more negative in autism group at the right hemisphere. Most consistent finding was that gamma induced by the nontarget stimuli was globally higher in autistic subjects compared to controls at all sites. This interaction for *Stimulus* (nontarget, target) × *Topography* (frontal, parietal) × *Group* (autism, control) was significant. Power density differences to target and nontarget stimuli revealed significant and reproducible effect of higher response to nontargets rather than target Kanizsa figures in the autism group (Sokhadze et al. 2009a).

Our findings of higher amplitude of ERP components (Sokhadze et al. 2009a, b; Casanova et al. 2012) and excessive gamma oscillations (Sokhadze et al. 2009a; Baruth et al. 2010, 2011) in response to nontarget items are in agreement with other studies noting that neural systems in the brain of autistic patients are often inappropriately activated (Belmonte and Yurgelun-Todd 2003a). Kemner et al. (1994) also reported that the visual N200 ERP component to novel distracters is larger when a person with autism is performing a task even when these novel stimuli are not relevant to the task in question. According to Belmonte and Yurgelun-Todd (2003a, b), perceptual filtering in autism occurs in an all-or-none manner with little specificity for the task relevance of the stimulus. Perceptual filtering may primarily depend on the control of general arousal rather than the activation of specific perceptual system. Since in many tasks requiring attention, persons with autism perform at close to normal levels despite generally high arousal and low selectivity, some compensatory mechanisms may operate at a higher stage of processing to sort out relevant stimuli from poorly discriminated background. One candidate mechanism was suggested as an active inhibition of irrelevant distracters that have passed through earlier filtering (Belmonte and Yurgelun-Todd 2003b). It is unsurprising that increased ratio of excitation/inhibition in key neural systems and high "cortical noise" have been considered as a core abnormality of autism (Casanova et al. 2003; Rubenstein and Merzenich 2003).

Our study showed very similar gamma activation pattern both to "target" and "nontarget" Kanizsa stimuli (Sokhadze et al. 2009a; Baruth et al. 2010, 2011). Furthermore, dipole source coherence analysis (Hoechstetter et al. 2004) of early evoked (40–150 ms) 40 Hz centered gamma responses to targets at the parietal sites (P3, P4) showed between three groups differences, specifically, higher hemispheric coherence coefficient values in attention deficit/hyperactivity disorder (ADHD) as compared to autism group (0.59 in ADHD vs. 0.38 in autism, $p = 0.003$).

The gamma frequencies, particularly those centered about 40 Hz, have been tied to visual, attentional, cognitive, and memory processes (Başar et al. 2001). As it was mentioned above, following a stimulus presentation during visual task, two gamma oscillations are typically noted: an early evoked oscillation and a late induced

oscillation (Başar et al. 2001). The evoked gamma oscillations typically occur within the first 100 ms after the onset of a stimulus and are locked in time from trial to trial. Because little variation is seen in the latency of the evoked gamma with changing stimulus type, it is believed that it may be a result of sensory processes. Conversely, induced gamma oscillations occur later, after 240 ms poststimulus, and vary in latency from trial to trial (Tallon-Baudry and Bertrand 1999). These variations occurring in time window typical for P300 ERP component may suggest that the induced gamma oscillations are related to higher cognitive processes (Tallon-Baudry 2003). Deviations from typical gamma-band activity have been reported in several studies on neurological disorders, including epilepsy, Alzheimer's disease, ADHD, and autism (Herrmann and Demiralp 2005).

Our study (Baruth et al. 2010) indicated that individuals with autism had a minimal difference in evoked gamma power between target and nontarget Kanizsa stimuli at all EEG channels of interest. In fact, evoked gamma power responses were slightly larger in response to nontarget Kanizsa stimuli relative to targets. In contrast the control group had a significantly higher evoked gamma power to target Kanizsa stimuli compared to nontarget Kanizsa stimuli showing clear differences in visual stimulus discrimination. Additionally, the control group showed a greater difference in evoked gamma power between frontal and parietal regions to all stimuli over the left hemisphere: controls had more frontal as compared to parietal gamma activity, while the autism spectrum disorder (ASD) group showed negligible topographic differences. These findings are similar to the findings of Grice et al. (2001) where individuals with autism did not show significant differences in frontal gamma activity during the processing of upright and inverted faces, whereas control subjects showed clear discriminative increases in frontal gamma activity when the faces were presented upright vs. inverted. These findings also correspond to our previous investigation (Sokhadze et al. 2009a) where we found positive differences in gamma oscillation power (i.e., 30–80 Hz, 0–800 ms poststimulus) between target and nontarget Kanizsa stimuli where it decreased, especially over the lateral frontal (F7, F8) and parietal (P7, P8) EEG sites, in adolescents and young adults with ASDs; this was mainly due to significant increases in gamma power at all recording sites, especially evoked gamma (i.e., ~100 ms) over frontal channels, to nontarget Kanizsa stimuli compared to controls. Our results indicate that in ASD evoked gamma activity is not discriminative of stimulus type, whereas in controls early gamma power differences between target and nontarget stimuli are highly significant.

There are a few plausible explanations as to why the gamma response does not allow for discrimination between stimuli in ASD. It is well known that ASD is associated with amplified responses to incoming sensory information. Studies suggest that the neural systems of individuals with ASD are over-activated (Belmonte et al. 2004a, b) and there is a lack of cortical inhibitory tone (Casanova et al. 2002a, b, 2006a; Rubenstein and Merzenich 2003). In a network that is over-activated and "noisy," local cortical connectivity may be enhanced at the expense of long-range cortical connections, and individuals with ASD may have difficulty directing attention. It may not be possible for them to selectively activate specific perceptual systems based on the relevance of a stimulus (i.e., target vs. nontarget).

Our previous findings investigating ERP during a visual novelty processing task further support the idea of difficulty discriminating task-relevant from irrelevant stimuli in ASD (Sokhadze et al. 2009b). Briefly, we found that subjects with ASD showed a lack of stimulus discrimination between target and nontarget stimuli compared to controls, and this was mainly due to significantly prolonged and augmented ERP components to irrelevant distracter stimuli over frontal and parietal recording sites. Early ERP components (e.g., P100, N100) were especially increased to irrelevant distracter stimuli in the ASD group indicating augmented responses at early stages of visual processing (i.e., ~100 ms). Early gamma components (i.e., evoked) are measured at the same time over the same cortical regions as these early ERP components. The very early burst of gamma activity between 80 and 120 ms found by Brown et al. (2005) and our findings of augmented evoked gamma (Sokhadze et al. 2009a) and early ERP responses (Sokhadze et al. 2009b) to task-irrelevant stimuli support the idea of disturbances in the activation task-relevant neuronal assemblies and the perceptual control of attention in ASD. Although we found significant group differences in relative evoked gamma power in processing relevant and irrelevant visual stimuli in this study, it is important to mention why we did not find significantly amplified relative evoked gamma power in the ASD group compared to controls. We attribute this to the fact that relative gamma-band power is calculated in reference to the entire EEG spectrum, and in ASD it has previously been shown that other frequency ranges are augmented as well (Dawson et al. 1995; Stroganova et al. 2007).

5.5 Language and Gamma Power and Coherence

Understanding of language requires integration of input of the different parts of information that are processed in different brain areas. It has been suggested that binding between different distributed parts of language processing neural network is implemented by synchronization and desynchronization of oscillatory neural activity (Singer 1999; Weiss and Müller 2003; Hald et al. 2006). Analysis of event-related changes in either power or phase coherence of EEG oscillations provides a window onto the processes of synchronization and desynchronization of neuronal populations (Tallon-Baudry and Bertrand 1999; Varela et al. 2001). Increased power and higher phase coherence between EEG recording sites is thought to reflect synchrony of activation and higher spatial co-activation of distributed neural systems that may reflect the transient formation of functional networks involved in language processing (Varela et al. 2001; Bastiaansen and Hagoort 2003; Penolazzi et al. 2009). For a proper analysis of oscillatory dynamics, one of the most analytic tools used is wavelet-based time-frequency analysis to quantify amplitude/power changes and/or event-related coherence analysis for quantifying changes in phase coherence between EEG electrodes or between dipole sources (Hoechstetter et al. 2004). Recent studies employing such techniques have clearly demonstrated that synchronous oscillations have functional significance during the execution of tasks

engaging a variety of cognitive operations, such as memory encoding and retrieval (Fell et al. 2001; Burgess and Ali 2002), working memory (Kahana et al. 1999; Jensen and Tesche 2002), face perception (Rodriguez et al. 1999), object detection (Tallon-Baudry and Bertrand 1999), and attentional processes (Klimesch 1999; Bastiaansen and Brunia 2001; Fries et al. 2001).

Recently, such studies are also being performed in the domain of language comprehension (Weiss and Rappelsberger 1996, 2000; Pulvermüller et al. 1999; Weiss et al. 2001; Bastiaansen et al. 2002a, b, 2005; Schack et al. 2003; Weiss and Müller 2003). Still, relatively little is known about synchronous oscillations and their possible functions during language comprehension. During the speech comprehension, different parts of the language processing system, such as auditory perception, phonological, morphological, syntactic, semantic, pragmatic, and prosodic analyses, have to be integrated in order to understand the meaning of the spoken sentences and to initiate appropriate response behavior. Large-scale synchronization seems particularly important with respect to distributed neuronal assemblies, which have to be integrated during complex cognitive processing (Bressler and Kelso 2001; Varela et al. 2001; Herrmann et al. 2004) and especially during language processing (Weiss and Rappelsberger 1996; Petsche and Etlinger 1998). In a study by Benasich et al. (2008), EEG gamma power was associated with attention measures in infants. A group of children with a family history of language impairment and thus at higher risk for language disorders showed consistently lower gamma over frontal regions than the well-matched controls with no such family history. The authors suggested that the emergence of high-frequency neural synchrony may be critical for cognitive and linguistic development, and children at risk for language impairments may lag in this process (Benasich et al. 2008). Further systematic studies on EEG coherence and language will elucidate and clarify the meaning and interpretation of previous findings linking EEG gamma and language.

5.6 Conclusions

Recently, there were several attempts at deriving an overarching metatheory of autism that have focused on a basic abnormality of neural connectivity (Belmonte et al. 2004a, b). This model is empirically based on lack of coordinated brain activity and abnormal "binding" in the brains of autistic patients that can be detected with EEG methodology, specifically using gamma oscillations (Brock et al. 2002; Brown 2005; Rippon et al. 2007). According to Baron-Cohen and Belmonte (2005), the combination of local sensory hyperarousal and low-level over-processing of incoming sensory stimuli concurrent with abnormalities of attention selectivity and focus may be a consequence of the over-connected low-level processing neural networks in ASDs. In such over-wired networks, signal is insufficiently differentiated from noise or task-irrelevant information, and as a result, information capacity is drastically reduced (Rubenstein and Merzenich 2003; Belmonte et al. 2004a, b; Casanova 2006). Higher-than-normal noise in cortical processes also affects normal

development of differentiated representations, because cortical response selectivity in space and time is a product of balanced inhibitory and excitatory processes. Such overrepresentation by non-differentiated systems could plausibly account, for example, for the strong aversive reactions to auditory, tactile, and visual stimuli that are commonly recorded in autistic individuals. The abnormal long-range neural connectivity model is suggested to explain deficits in high-level complex information processing functions where rapid and integrated operation of many separate neural systems is required (Minshew et al. 1997; Welchew et al. 2005). In the autistic brain, high local connectivity may develop along with deficient long-range connectivity.

In recent years, neuropathological studies of autism have revealed abnormalities in several brain regions. Changes in brain size with widespread increases in both gray and white matter volumes suggest that the underlying pathology in autism consists of widely distributed histological abnormalities. The available neuropathological and structural imaging data suggest that autism is the result of a developmental lesion capable of affecting normal brain growth. One possible explanation for this is the recent finding of minicolumnar abnormalities in autism, in particular demonstration of minicolumns of reduced size and increased number in the autistic brain (Casanova et al. 2002a, b, 2006a, b). The increased number of minicolumns reported in autism suggests a possible disruption during the earlier stages of neurodevelopment in the brain of an autistic patient. Furthermore, a minicolumnar abnormality may translate difficulties in the integration of information into a delay in language acquisition. In all, minicolumnar abnormalities may incapacitate a patient as a social being by distorting elements of the child's biopsychological experience.

The modular arrangement of the cortex is based on the cell minicolumn: a self-contained ecosystem of neurons and their afferent, efferent, and interneuronal connections (Mountcastle 2003). Our preliminary studies indicate that minicolumns in the brains of autistic patients are narrower, with an altered internal organization (Casanova 2006). More specifically, their minicolumns reveal less space for inhibitory local circuit projections. A defect in these GABAergic fibers may correlate with the increased prevalence of seizures among autistic patients. Based on the descriptions given thus far, it is possible to propose a disruption of the normal balance between excitation and inhibition in the columnar organization of autistic patients. In this regard, a series of noteworthy studies report that both children and adults with autism were superior to a control group in their ability to discriminate novel, highly similar stimuli (Plaisted et al. 2003). Autistic children also have a superior ability in discriminating display items in visual search tasks; such enhanced discrimination in autism results from low-level perceptual processing of incoming stimuli, and this is called the bottom-up approach.

Analysis of high-frequency EEG oscillations in patients with autism may provide additional information about potential neural deficits in autism. Abnormalities in these mechanisms have been associated with binding problems (the co-activation of neural assemblies), which may be present in both autism and schizophrenia (Grice et al. 2001; Brock et al. 2002). Oscillatory activity in the gamma band of the EEG has been related to Gestalt perception and to cognitive functions such as attention, learning, and memory (Kaiser 2003). Electrophysiological studies show strong

evidence that synchronized cortical activity in the gamma-frequency range could be a correlate of feature binding to form a single coherent percept. Binding of widely distributed cell assemblies by synchronization of their gamma-frequency activity is thought to underlie cohesive stimulus representation in the human brain (Kahana 2006). According to this assumption, changes in gamma EEG activity have been considered indicators of processing of Gestalt-like patterns (von Stein et al. 1999; Herrmann and Mecklinger 2000, 2001).

The "weak central coherence" (Frith and Happé 1994) in autism could result from a reduction in the integration of specialized local networks in the brain caused by a deficit in temporal binding (Brock et al. 2002). Visual and auditory perception anomalies may be attributed to a reduced coherence and synchrony of gamma activity between networks processing local features and thus explain some of the language deficits, executive dysfunctions, and other impairments in social communication in autism. The inability to reduce gamma activity according to Brown (2005) would lead to the inability to decide which event requires attention when there are multiple choices. Excessive gamma can therefore be linked to a reduction in the ability to focus attention. The "temporal binding deficit" hypothesis of autism (Brock et al. 2002; Rippon et al. 2007) suggests that many features of autism, such as superiority in processing detail (local processing) and disadvantages in global processing, can be explained by a failure of binding between cortical areas. Analysis of evoked and induced EEG gamma oscillation can therefore significantly contribute to understanding the neurobiological nature of core autism symptoms and definitely warrant further rigorous investigations.

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Biography

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Joshua Baruth graduated from the Department of Anatomical Sciences and Neurobiology at the University of Louisville School of Medicine. Joshua's research has focused primarily on the treatment of autism spectrum disorders with transcranial magnetic stimulation. He received his B.A. in classical languages and premedicine from the University of Kansas in 2005 and his master's degree in anatomical sciences and neurobiology from the University of Louisville in 2009 and his PhD in 2010. He is currently at Mayo Clinic in Minnesota as a postdoc fellow.

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Estate M. Sokhadze received a Ph.D. in human physiology in 1988 (Novosibirsk, Russia). He completed a postdoctoral fellowship in psychopharmacology at Wake Forest University in 2001–2003 and postdoctoral training in cognitive neuroscience at Rice University in 2004. Currently, Dr. Sokhadze is an associate professor of Psychiatry and Behavioral Sciences at the University of Louisville and is a director of the Evoked Potential Lab at Cognitive Neuroscience Labs. His research interests include application of dense-array EEG/ERP brain mapping, neurofeedback, TMS, and other applied psychophysiological techniques in psychiatric research. Specific psychopathology areas of interest are substance abuse, PTSD, autism, ADHD, conversion disorder, bipolar disorder, and comorbid mental conditions. He has more than 25 years of experience in applied psychophysiology and clinical neurosciences.

Chapter 6
Neurofeedback for Autistic Disorders: Emerging Empirical Evidence

Robert Coben

6.1 Introduction

Autistic spectrum disorders are a heterogeneous group of pervasive developmental disorders including autistic disorder, Rett disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger's disorder. Children with ASD demonstrate impairment in social interaction, verbal and nonverbal communication, and behaviors or interests (American Psychiatric Association 2000). ASD may be comorbid with sensory integration difficulties, mental retardation, or seizure disorders. Children with ASD may have severe sensitivity to sounds, textures, tastes, and smells. Cognitive deficits are often associated with impaired communication skills (National Institute of Mental Health; NIMH, 2006). Repetitive stereotyped behaviors, perseveration, and obsessionality, common in ASD, are associated with executive deficits. Executive dysfunction in inhibitory control and set shifting have been attributed to ASD (Schmitz et al. 2006). Seizure disorders may occur in one out of four children with ASD, frequently beginning in early childhood or adolescence (National Institute of Mental Health; NIMH, 2006).

Autistic disorder includes the following triad of symptoms: (1) impaired social interaction, failure to develop peer relationships, or lack of initiating spontaneous activities; (2) deficits in communication including delay in or lack of spoken language, inability to initiate or sustain conversation with others, stereotyped repetitive use of language, or idiosyncratic language; and (3) restricted repetitive and stereotyped behavior, interests, inflexible adherence to routines or rituals, and repetitive motor patterns (e.g., hand or finger flapping or twisting) (American Psychiatric Association 2000).

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Individuals with Asperger's disorder frequently have high levels of cognitive functioning, engage in literal pedantic speech, experience difficulty comprehending implied meaning, exhibit problems with fluid movement, and manifest inappropriate social interactions. Pervasive developmental disorder-not otherwise specified (PDD-NOS) reflects deficits in language and social skills, which do not meet the criteria of other disorders. In contrast, persons with childhood disintegrative disorder and Rett disorder both have normal periods of early development followed by loss of previously acquired skills. Common features among all these conditions include communication and social skill deficits. There is considerable variability in terms of onset and severity of symptomatology within the autistic spectrum of disorders (Siegel 1996; Attwood 1998; Hamilton 2000; Sicile-Kira 2004; McCandless 2005).

Research reviewing the epidemiology of autism (Centers for Disease Control and Prevention 2009) reported between 1 in 80 and 1 in 240 children in the United States diagnosed with the disorder. A report of just 3 years ago (Centers for Disease Control and Prevention 2009) suggested a prevalence of 1 in 110 and as high as 1 in 70 boys. In their most recent report, the CDC (2012) suggests that the rate has risen to 1 in 88. ASDs are five times more likely in boys for which it is seen in 1 out of 54 male children. According to Blaxill (2004), the rates of ASD were reported to be <3 per 10,000 children in the 1970s and rose to >30 per 10,000 in the 1990s. This rise in the rate of ASD constituted a tenfold increase over a 20-year interval in the United States. With increased prevalence comes a need to design and empirically validate effective treatments for those impacted by autistic disorders.

Research studies utilizing electroencephalogram (EEG) and single photon emission computed tomography (SPECT) have provided evidence for a neuropathological basis of ASD. A review of numerous EEG studies reported the rate of abnormal EEGs in autism ranged from 10 % to 83 %, while the mean incidence was 50 %. Atypical EEGs often predict poor outcomes for intelligence, speech, and educational achievement (Hughes and John 1999). In a more recent review of research, Rippon et al. (2007) proposed a model of reduced connectivity between specialized local neural networks and overconnectivity within isolated neural assemblies in autism. Disordered connectivity may be associated with an increased ratio of excitation/inhibition in key neural systems. Anomalies in connectivity may be linked to abnormalities in information integration. In SPECT scans of children with autism, abnormal regional cerebral blood flow in the medial prefrontal cortex and anterior cingulate gyrus was related to impaired communication and social interaction, while altered perfusion in the right medial temporal lobe was associated with the obsessive desire for sameness (Ohnishi et al. 2000). Children with autism commonly display executive functioning deficits in planning, cognitive flexibility, and inhibition. These executive deficits are associated with dysfunctional integration of the frontal lobes with other brain regions and thus also impact upon social, behavioral, and cognitive function (Hill 2004).

Functional neuroimaging studies have also linked social cognition dysfunction and language deficits in autism to neural substrates (Pelphrey et al. 2004; Welchew et al. 2005). During a sentence comprehension test, individuals with autism showed less functional connectivity between Broca's and Wernicke's areas relative to a

control group, suggesting a lower degree of information organization and neural synchronization during language tasks (Just et al. 2004). A review of neuroimaging studies has found key brain structures including the amygdala, superior temporal sulcus region, and fusiform gyrus to function differently in individuals with autism than in controls (McAlonan et al. 2005).

Parents of children with ASD select many different methods of treatment, with an average of seven different therapies being utilized (Green, Pituch, Itchon, Choi, O'Reilly, and Sigafoos, 2006). Speech therapy (70 % of parents) was the most commonly selected treatment, followed by psychopharmacological treatment (52 % of parents). Other treatments included visual schedules (43 %), sensory integration (38 %), and applied behavior analysis (36 %). Special diets were implemented by 27 % of parents and 43 % utilized vitamin supplements. While there may be some benefit to these treatments, many do not lead to long-lasting changes and/or have risks associated with their implementation. The potential benefits and risks of the major treatments for ASD are summarized below.

6.2 Treatments Often Used for ASDs

Other than neurofeedback, the most common treatments used for these children include applied behavior analysis (ABA), pharmacotherapy, special diets, vitamin supplements and enzymes, chelation, and hyperbaric oxygen therapy. Applied behavior analysis (ABA), a form of behavior modification, is the method of treatment with the most empirical support for treating ASD. The goal of this therapy is to improve social interaction, behavior, and communication (Bassett et al. 2000). ABA is firmly based on the principles of operant conditioning and measures small units of behavior to build more complex and adaptive behaviors through reinforcement. Typically, imitation, attention, motivation, and compliance are targeted early (Couper 2004). Efficacy has been demonstrated across multiple studies with variations on the technique (Schopler and Reichler 1971; Lovaas et al. 1973; Ozonoff and Cathcart 1998; Herbert et al. 2002; Ben-Itzchak and Zachor 2007) with follow-up studies showing ongoing improvements as a result (McEachin et al. 1993). Unfortunately, not all ABA studies have had such positive outcomes (Anderson, Avery, DiPietro, Edwards, and Christian, 1987).

In their clinical practice guidelines report, the New York State Department of Health Early Intervention Program recommended that ABA and other behavioral interventions be included in the treatment of autism. They specify that intensive behavioral programs should include a minimum of 20 h of intervention with a therapist per week. Furthermore, the guidelines state that parents should be included in the intervention and that they be trained in the use of behavioral techniques to provide additional instruction at home with regular therapist consultation. Although promising, intensive behavioral programs are costly and require extensive time on the part of the therapist as well as the family, and debates are ongoing about who should pay for such services (Couper 2004).

Although behavior therapy improves social, cognitive, and language skills, a year or more of intensive training has been used in most research studies that have demonstrated improvement. Furthermore, a strong commitment by parents to complete therapeutic programs is necessary to achieve positive outcomes. While behavioral treatment methods show the most empirical support to date, there remains a need for additional therapies, which may be more easily administered and used in conjunction with the behavioral methods described. It is important to note that though research has been promising, there has been great variability between studies in their results and outcome measures have often been questionable (e.g., IQ scores, returning to regular classrooms). And this approach appears to be more effective with those who are higher functioning (i.e., higher IQ), meaning that lower functioning individuals are often left out, even though they are perhaps in greatest need of treatment.

Pharmacological interventions have also been utilized to treat individuals with ASD. A study conducted at the Yale Child Study Center found that 55 % of a group of 109 individuals with a PDD were taking psychotropic medication, with 29.3 % taking more than one medication (Martin, Scahill, Klin, and Volkmar 1999). The most common medications were antidepressants (32.1 %), followed by stimulants (20.2 %) and neuroleptics (16.5 %). The objectives of psychopharmacological treatment for autism include decreasing the core symptoms of autism, decreasing anxiety and overfocus, improving social skills, reducing aggressive self-injurious behavior, increasing the effects of other interventions, and improving the quality of life for the child and their family. There is no single medication known to be beneficial to all children with ASD nor that has specifically been developed for individuals with autistic spectrum disorder.

Psychostimulant medications are often used with children who are autistic due to its success in the treatment of ADHD (Jensen et al. 2007). Despite this, stimulant use in children who are autistic remains controversial and largely unproven in terms of efficacy (Research Units on Pediatric Psychopharmacology Autism Network 2005). A newer class of neuroleptic, referred to as atypical antipsychotics, reportedly improves social interaction and decreases aggression, irritability, agitation, and hyperactivity (Barnard et al. 2002). They have fewer extrapyramidal adverse side effects than haloperidol and thioridazine. However, most children experience a substantial weight gain within the first months of treatment (Committee on Children with Disabilities 2001). Risperidone and Abilify are the only drugs approved by the FDA to treat the symptoms (irritability) of autism. A recent meta-analysis of three randomized controlled trials found that the drug was effective in treating the symptoms of irritability and aggression (Jesner et al. 2007). The authors concluded that although risperidone may be beneficial, its use must be weighed against its adverse effects, most notably weight gain, and that long-term follow up is needed prior to determining its efficacy in clinical practice. The long-term effects of risperidone are estimated at 1 year (Zuddas et al. 2000) with a relapse rate of 12.5–25 % (Research Units on Pediatric Psychopharmacology Autism Network 2005; Troost et al. 2005). Santangelo and Tsatsanis (2005) reported that there are currently no drugs that produce major improvement in the core social or pragmatic language deficits in autism, although several have limited effects on the behavioral features of the disorder.

The use of SSRI agents for the treatment of repetitive, stereotypical, and perseverative behaviors has also been explored (McDougle et al. 1995; Geller et al. 2001). Findings from such studies have been mixed at best (Cook et al. 1992; Hollander et al. 2005). While some studies report "success," responders often include from 49 to 69 % of the samples (McDougle et al. 1996, 1998; DeLong et al. 2002; Owley et al. 2005). In other studies, the positive response rate is significantly lower than this (McDougle et al. 2000; Couturier and Nicolson 2002; Martin et al. 2003). Based on the research cited, it appears that the limited benefits of psychopharmacology come at the cost of side effects and rebound of aggressive behavior when medication is discontinued. Furthermore, these drugs appear to be only treating certain symptoms and typically not the core symptoms of ASD. Many children require multiple medications to improve their symptoms, and often the benefits do not outweigh the side effects. In addition to patients responding to highly variable doses, the majority of studies reviewed indicate that not all children with ASD respond to these various medications, and there is no good explanation for why some are considered responders and some are not. In summary, the research published thus far suggests that some medications may be helpful in managing some of the behavioral disturbances seen in autism.

Research has suggested that individuals with autism may not properly metabolize the proteins in casein (dairy) and gluten (wheat and related grains) resulting in an opioid effect on the brain as they enter the bloodstream (Reichelt, 2001). Use of a gluten–casein-free diet has been shown to lead to positive outcomes in some children with autism (Knivsberg et al. 2002; Cade et al., 1999; Reichelt and Knivsberg, 2003). However, more recently, Elder et al. (2006) conducted a rigorous double-blinded controlled trial of the GFCF diet in autism. Fifteen (12 boys, 3 girls) children with ASD between the ages of 2 and 16 were studied over the course of 12 weeks. The researchers reported no significant differences between groups on their primary measure, the Childhood Autism Rating Scale, while parents reported improvement in their children. The researchers noted that the children were quite heterogeneous (which may have masked any group differences) and noted the relatively small sample size. One of the major problems with the GFCF diet is that it may lead to reduced bone cortical thickness (Hediger et al. 2008). Indeed, in this study, boys between the ages of four and eight who were autistic showed an 18.9 % deviation in metacarpal bone cortical thickness, which was nearly twice that of boys on minimally restricted or nonrestricted diets. Furthermore, the GFCF diet may induce nutritional imbalances by limiting the foods that may be eaten. It has also been shown to increase the risk of becoming overweight/obese (Mariani et al. 1998).

Vitamin supplements and enzymes have been proposed as another treatment for autistic-related symptoms. One supplement that has generated a great deal of interest as a treatment for autism is the gastrointestinal hormone secretin. After receiving much heated attention in the media, a comprehensive review of research studies utilizing secretin to treat autism was conducted by Esch and Carr (2004). Seventeen quantitative studies were reviewed, encompassing approximately 600 children, ages 2–15, and 12 adults with ASD. Only one of the studies reviewed found a causal relationship between secretin administration and amelioration of autistic symptoms across various treatment variables (type of secretin, dosage potency, frequency), observation

times, and participant characteristics (e.g., GI status, severity of ASD, age, history of medication use). Twelve of the thirteen placebo-controlled studies reviewed obtained negative results. Despite the lack of empirical support for secretin, parents of autistic children continue to seek out secretin treatment from their physicians (Esch and Carr 2004). The reviewers attempted to explain this by the media attention that secretin received early on, coupled with the fact that parents of these children are often desperate to find a treatment for this debilitating condition. In addition to secretin, it has been suggested that the consumption of omega-3 fatty acids may have a positive effect on the symptoms of autism (Amminger et al. 2007). These highly unsaturated fatty acids are essential for normal brain development and functioning (Wainwright 2002), and some studies have found fatty acid deficiencies in children who are autistic (Bell et al. 2000; Vancassel et al. 2001; Bell et al. 2004). Amminger and colleagues (2007) recently completed a double-blind, randomized controlled trial of omega-3 fatty acid supplementation in children who were autistic. They found that with administration of 1.5 g/day, the treatment group showed no significant change in hyperactive behaviors including disobedience, distractibility, and impulsivity, relative to the control group. Potential limitations to this study include that it was conducted with only 12 subjects, and preselection of these subjects was based on high irritability scores based on the Aberrant Behavior Checklist (Aman et al. 1985).

Anecdotal reports that methyl-B_{12} (methylcobalamin) injections may improve the symptoms of autism have been plentiful; however, there have been very few controlled research studies to support the efficacy of this treatment. The only published study found by the authors was an open trial of methyl-B_{12} conducted in Japan with 13 children with autism, ranging from 2 to 18 years of age (Nakano et al. 2005). Dosages of 25–30 g/kg/day were administered for between 6 months and 25 months. The authors found a significant increase in the intelligence and developmental quotients, as well as improvement on the Childhood Autism Rating Scale (Schopler, Reichler, DeVellis, and Daly, 1980). Even after the children were divided into subgroups based on age and intelligence, these effects did not diminish. This was not a controlled study, however. In contrast, a preliminary report of a double-blind crossover study presented at the American Academy of Child and Adolescent Psychiatry conference revealed no significant benefits in the 14 patients in their study after 3 months (Deprey et al. 2006). Specifically, there were no differences between the methyl-B_{12} injections and the placebo on the Clinical Global Impression Scale Improvement, Peabody Picture Vocabulary Test, or Social Communication Questionnaire verbal results.

A controversial theory to explain the increase in incidence of ASDs over the past 30 years is that it is related to environmental factors such as exposure to heavy metals (Bradstreet et al. 2003), mercury (Hg) in particular. The medical literature indicates that autism and Hg poisoning have numerous similarities in their symptom profiles, including psychiatric disturbances, speech, language, and hearing difficulties, sensory impairment, and cognitive difficulties (Bernard et al. 2000). In autism, heavy metal toxicity seems to occur from a decreased ability to excrete heavy metals (Adams et al. 2009). Because of this, some health-care providers are performing chelation therapy, which utilizes dimercaptosuccinic acid (DMSA) to clear the body of mercury and other toxic metals.

Results of a study by Holmes (2001) suggest that chelation therapy may be effective only for young children with autism (under age six), with minimal benefit for older children and adolescents (Kirby 2005). Recently, Adams et al. (2009) reported the results of a 2-phase study intended to determine the efficacy of DMSA/glutathione in treating children with autism. Overall, there were rated improvements in 3 of every 4 children with 11 % showing a worsening of symptoms. Chelation therapy is considered by some to be a risky treatment, and there have even been reports of death following chelation therapy in autism (Sinha et al. 2006).

Direct treatment of brain anomalies in autism has also been pursued with the use of hyperbaric oxygen therapy (HBOT). Among other brain abnormalities that have been identified, numerous studies using PET and SPECT have shown cerebral hypoperfusion in autism (George et al. 1992; Mountz et al. 1995; Ohnishi et al. 2000; Starkstein et al. 2000; Zilbovicius et al. 2000), leading to the hypothesis that HBOT may be beneficial in the treatment of autism (Rossignol and Rossignol 2006). HBOT involves the inhalation of 100 % oxygen in a pressurized chamber, usually above one atmosphere absolute (ATA). It has been shown that HBOT can lead to improved functioning in various neurological populations that show cerebral hypoperfusion including stroke (Nighoghossian et al. 1995), cerebral palsy (Montgomery et al. 1999), chronically brain injured (Golden et al. 2002), and even a teenage male with fetal alcohol syndrome (Stoller 2005). It has been suggested that the increased oxygen delivered by HBOT could counteract the hypoxia caused by hypoperfusion and lead to a reduction in symptoms of autism. Preliminary support for this treatment was reported by Rossignol and Rossignol (2006). While a study by Rossignol et al. (2007) showed empirical support for the possible benefits of HBOT for autistic children, another study (where parents were blinded to the treatment) by Granpeesheh et al. (2010) showed no significant benefits.

In summary, this review of the autism treatment literature reveals there are no treatments, except possibly behavior therapy, that have been well validated or that have exhibited favorable long-term results. In addition, many forms of intervention include the possibility of adverse effects, require long-term use, or were not developed specifically for autistic spectrum disorders. Neurofeedback represents an alternative that may have the potential to decrease symptomatology on a long-term basis with little risk of harm.

6.3 Neurofeedback for ASD

Neurofeedback is designed to use sophisticated computer technology to train individuals to improve poorly regulated brain-wave patterns. In EEG biofeedback, information regarding brain-wave activity is fed to a computer that converts this information into game-like displays that can be auditory, visual, or both. During a typical session, EEG electrodes (which measure brain waves) are placed on the scalp and earlobe(s). Individuals instantly receive feedback about the amplitude and/or synchronization of their brain waves and learn to improve their brain-wave

Table 6.1 EEG frequency bands [adapted from Demos (2005) and Thompson and Thompson (2003a, b)]

Name	Frequency	Normal occurrence	Significance
Delta	0.5–3.5 Hz	Deep sleep and infants	Sign of significant brain dysfunction, lethargy/drowsiness, or cognitive impairment
Theta	4–7.5 Hz	Young children, drowsiness, some aspects of learning	Slowing often related to attention/ cognitive impairments, internal focus
Alpha	8–13 Hz	Eyes closed, relaxation, self-awareness	Excessive alpha during demand states can be a sign of difficulties with learning, emotional stability, relating to the environment, or others
Beta	13–30 Hz	Fast activity associated with alertness and activity	Excessive beta is often associated with anxiety, irritability, and poor integration
Gamma	>30 Hz	May be associated with problem solving and memory consolidation	Unknown

functioning. The only way to succeed at the games involved is for children to control and improve their brain-wave patterns (following an operant-conditioning paradigm). In research and clinical treatment for children with ADHD, this conditioning process has resulted in improvements that have persisted for up to 5–10 years or more (e.g., Lubar 1995).

Individuals who participate in EEG biofeedback learn to inhibit brain-wave frequencies that may produce negative symptoms and enhance specific frequencies that produce positive results. Table 6.1 displays the typical EEG brain-wave frequency bands and lists their normal occurrences and respective significance [information adapted from resources contained in Demos (2005) and Thompson and Thompson (2003a, b)]. Within these general frequency bands, there may also be more detailed breakdowns of EEG activity. For example, mu-rhythm abnormalities are associated with excesses in the alpha-frequency band and have a characteristic morphologic and topographic distribution (Coben and Hudspeth 2006). Subdivisions of beta power have also been presented and related to clinical characteristics (Rangaswamy et al. 2002).

Individuals with poorly regulated cortical activity can learn to develop a fluid shift in brain waves to meet task demands utilizing neurofeedback. Through the process of operant conditioning, this treatment modality can result in improvement of brain-wave patterns as well as behavior. These changes in EEG patterns have been shown to be associated with regulation of cerebral blood flow, metabolism, and neurotransmitter function (Lubar 1997).

Neurofeedback is a noninvasive treatment with no known significant or lasting negative side effects that has been shown to enhance neuroregulation and metabolic function in ASD (Coben and Padolsky 2007). Positive neurofeedback treatment outcomes are often achieved over the course of several months, in contrast to behavior therapy, which often takes a year or more of intensive training. Furthermore, the therapeutic treatment outcomes of neurofeedback training with individuals with

ADHD (increased attention, reduced impulsivity, and hyperactivity) have been reported to be maintained over time and not reverse after treatment is withdrawn as in drug therapy and diet therapy (Tansey 1993; Linden et al. 1996; Monastra et al. 2005; Lubar, Swartwood, Swartwood, and O'Donnell, 1995).

Over 30 years of research on using neurofeedback to treat ADHD has consistently shown that it leads to improvements in attention, impulsivity, hyperactivity, and IQ (see Monastra et al. 2005, for a review and analysis). This success was the foundation for the emergence of using neurofeedback with ASD.

6.3.1 QEEG Evaluation and Autistic Spectrum Disorder

Quantitative electroencephalographic (QEEG) evaluation or "brain mapping" is an assessment procedure designed to pinpoint anomalies in brain function (Hammond 2005). QEEG analyses measure abnormalities, instabilities, or lack of proper communications pathways (connectivity) necessary for optimal brain functioning. QEEG maps, collected using 19 electrodes based on the international 10–20 system (Jasper 1958), reflect quantitative analyses of EEG characteristics of frequency, amplitude, and coherence during various conditions or tasks. These data can be statistically compared to an age-matched normative database to reveal a profile of abnormalities. Such regions and aspects of dysfunctional neurophysiology may then be targeted specifically through individualized neurofeedback protocols.

QEEG analyses are conducted to assess underlying neurophysiological patterns related to the symptoms and challenges of children with ASD. In addition, assessment of the raw EEG can be used to evaluate neurological abnormalities such as seizure disorders, which are common in children with autism. QEEG data are important for developing the most individualized, specific, and successful neurofeedback protocols for patients with ASD (Coben and Padolsky 2007; Linden 2004).

Coben et al. (2013) identified five relative power subtypes in individuals with autism. However, they noted that many types of dysfunction overlap in people with autism, and most reveal a combination of findings. In over 83 % of the individuals with autism, connectivity anomalies could be identified when compared to the normative group. Coben and Myers (2008) used QEEG multivariate connectivity data to develop a typology of autism connectivity patterns including (1) patterns of hyperconnectivity across bilateral frontotemporal regions and between left hemisphere locations and (2) hypoconnectivity involving orbitofrontal, frontal to posterior, right posterior, or left hemisphere sites. A pattern of hypoconnectivity that underlies a mu-rhythm complex was identified as well.

6.3.2 Neurofeedback: Case Studies, Case Series, and Group Pilot Studies

There have been numerous case and group pilot studies conducted with clients diagnosed with autistic spectrum disorders. In general, these studies have shown

that neurofeedback improved symptomatology and these improvements were maintained at follow-up. For a more thorough review of these, please see Coben et al. (2010b).

6.3.3 Controlled-Group Studies of Neurofeedback for ASD

There have been two approaches to the research done related to neurofeedback and ASD. Kouijzer and her colleagues have researched the effects of power training and Coben and his colleagues the effects of coherence training. The first study of Kouijzer and colleagues (2009b) investigated the effects of neurofeedback in children with autism. It included 14 children from 8 to 12 years old with a pervasive developmental disorder—not otherwise specified (PDD-NOS)—diagnosis. Excluded were children with an IQ score below 70, children using medication, and children with a history of severe brain injury or comorbidity such as ADHD or epilepsy. Participants were divided into treatment and wait-list control group according to the order of applying. During baseline (Time1), all participants were evaluated using QEEG and a range of executive function tasks, and parents completed behavior questionnaires (CCC and Auti-R). After neurofeedback training (Time2), or a comparable time interval for the wait-list control group, QEEGs and data on executive functions and social behavior were re-collected. One year after ending treatment (Time3), follow-up data including QEEGs, executive function tasks, and behavior questionnaires were collected in the treatment group. Participants in the treatment group had neurofeedback training twice a week, until 40 sessions were completed. In each session, participants were rewarded when inhibiting theta power (4–8 Hz) and increasing low beta power (12–15 Hz) at scalp location C4 according to a protocol including seven 3 min intervals of neurofeedback training separated by 1 min rest intervals. After 40 sessions of neurofeedback, 70 % of the participants in the treatment group had effectively decreased theta power and increased low beta power. Repeated measures MANOVA on the executive functions data collected at Time1 and Time2 revealed a significant interaction between treatment and control group, indicating improvement of participants in the treatment group on tasks measuring attention skills, cognitive flexibility, set shifting, concept generation/inhibition, and planning. Using repeated measures MANOVA to compare questionnaire data collected at Time1 and Time2 revealed a significant interaction effect between treatment and control group, indicating improvement in nonverbal communication and general communication. Time2 Auti-R questionnaire data evaluating changes in behavior over the last 6 months showed significant improvement in social interactions, communication skills, and stereotyped and repetitive behavior for the treatment group, but not for the control group.

In a second study by Kouijzer and colleagues (2010), several methodological improvements were implemented to better identify the effects of neurofeedback. A randomized wait-list control group design was used, and the study was conducted at the schools of the participants ($n = 20$). Participants were 8–12 years old and

had diagnoses of autism, Asperger's disorder, or PDD-NOS. Participants in the treatment group had 40 individual neurofeedback sessions using an individualized treatment protocol based on an initial QEEG. However, all treatment protocols included theta inhibition at fronto-central scalp locations. Treatment response was evaluated by QEEG measures taken during rest and task conditions, a range of executive function tasks, and social behavior questionnaires filled out by parents and teachers. All data were collected before (Time1) and after treatment (Time2) and at 6 months follow-up (Time3).

Results of the study showed that 60 % of participants decreased theta power within 40 sessions of neurofeedback. Additionally, repeated measures MANOVA on QEEG data revealed a significant interaction between treatment and control group, indicating a decrease in theta power in the treatment group in two out of four QEEG conditions. Repeated measures MANOVA on Time1 and Time2 executive function data showed a significant interaction between treatment and control group for cognitive flexibility, indicating improvement in cognitive flexibility in the treatment group compared to the control group. Repeated measures MANOVA showed a significant interaction effect for social interactions and communication skills, indicating that parents of participants in the treatment group reported significant improvement in social interactions and communication skills, whereas less or no improvement was reported by parents of children in the control group.

Coben and his colleagues began researching the effects of coherence/connectivity training on autistic symptoms about 6 years ago. Coben and Padolsky (2007) published a study investigating the effects of neurofeedback treatment for autistic disorders. The study included 49 children on the autistic spectrum, with 37 participants receiving QEEG connectivity-guided neurofeedback and 12 participants in a wait-list control group. Treatment included 20 sessions performed twice per week. The control group was matched for age, gender, race, handedness, other treatments, and severity of ASD. According to the parents, there was an 89 % success rate for neurofeedback and an average of 40 % reduction in core ASD symptomatology. There were significant improvements on neuropsychological measures of attention, visual–perceptual skills, language functions, and executive functioning. Importantly, reduced cerebral hyperconnectivity was associated with positive clinical outcomes, and in all cases of reported improvement, positive outcomes were supported by neurophysiological and neuropsychological assessment.

Mu-rhythm abnormalities are a sign of mirror neuron dysfunction, which is thought to be the case in many children with autism (Oberman et al. 2005). In two studies focused on reducing abnormal mu rhythms in children with autism, Pineda and Hecht (2009) found that according to parents, participants showed a small but significant reduction in symptoms but increased ratings of sensory-cognitive awareness. In another study related to mu rhythms, Coben and Hudspeth (2006) studied fourteen children with ASD who were identified as having significantly high levels of mu activity and a failure to suppress mu during observational activity. They all received assessment-guided neurofeedback, with a strong focus on aspects of mu power and connectivity. The participants were nonrandomly assigned to an interhemispheric bipolar training ($n = 7$) or a coherence training ($n = 7$) group designed to

increase connectivity between central regions and the peripheral frontal cortex. All patients were given neurobehavioral and neuropsychological testing and QEEG assessment. Both groups of patients improved significantly on neurobehavioral and neuropsychological measures. However, only in the coherence training treatment group was mu activity significantly reduced. Increased coherence was associated with diminished mu and improved levels of social functioning. Lastly, Coben (2007) conducted a controlled neurofeedback study focused on intervention for prominent social skill deficits based on a facial/emotional processing model. Fifty individuals with autism were included in these analyses, and all had previously had some neurofeedback training. All patients underwent pre- and post-treatment neuropsychological, QEEG, and parent rating scale assessments. Twenty-five individuals were assigned to either an active neurofeedback or a wait-list control group, in a randomized fashion. The two groups were matched for age, gender, race, handedness, medication usage, autistic symptom severity, social skill ratings, and visual–perceptual impairment levels. Neurofeedback training was QEEG connectivity guided and included coherence training (along with amplitude inhibits) between maximal sights of hypocoherence over the right posterior hemisphere. The group that received the coherence training showed significant changes in symptoms of autism, social skills, and visual–perceptual abilities such that all improved. Regression analyses showed that changes in visual–perceptual abilities significantly predicted improvements in social skills. EEG analyses were also significant, showing improvements in connectivity and source localization of theta power related to brain regions (fusiform gyrus, superior temporal sulcus) associated with enhanced visual/facial/emotional processing.

In the seven controlled-group studies that have been completed, a total of 214 individuals with autism have been studied and positive results reported in each study. These findings have included positive changes as evidenced by parental report, neuropsychological findings, and changes in the EEG (Coben 2007). Both Coben and Padolsky (2007) and Yucha and Montgomery (2008) have viewed these data as demonstrating a level of efficacy of "possibly efficacious" based on the standards put forth by the Association for Applied Psychophysiology and Biofeedback (AAPB 2006). Added to these initial findings of efficacy is preliminary evidence that the effects of neurofeedback on the symptoms of autism are long-lasting (1–2 years) (Coben 2009; Kouijzer et al. 2009a). While these findings are initially encouraging, there are many limitations that prevent firm conclusions to be drawn from the data collected thus far.

First, these studies have largely included nonrandomized samples. It is possible that an unknown selection bias exists which could have impacted the findings. Second, none of these studies have included participants or therapists/experimenters who were blind to the condition. Knowledge of group placement could have impacted the findings such that those in treatment (and their parents) would be prone to report significant changes. Third, there has been no attempt to control for placebo effects, attention from a caring professional, or expectations of treatment benefit. A randomized, double-blinded, placebo-controlled study is clearly needed to further demonstrate efficacy.

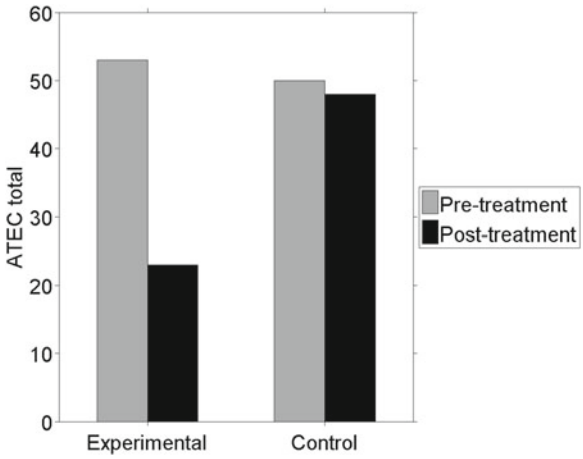

Fig. 6.1 Pre- and post-treatment ATEC scores

In terms of generalization of these findings to the larger population of individuals who are autistic, very young children and adults have not been well represented in these group studies. Lastly, there is the question of whether neurofeedback may be applicable to persons who are lower functioning or who have more severe symptoms associated with autism. These populations also should be the focus of future investigations.

6.3.4 Efficacy of Connectivity-Guided Neurofeedback for Autistic Spectrum Disorder

Recently, Coben (2009) presented on a study of the effects of an entire course of connectivity-guided neurofeedback treatment on autistic children. This included 110 subjects on the autistic spectrum, with 85 in the experimental and 25 in the control (wait-list) group. The mean age of these subjects was 9.7 years (range 4–20 years). Seventy-seven percent of these subjects were not on medication at the time, while 14 % were on one medication, 7 % on two medications, and 1 % on three medications. The mean IQ of this group was 93 (range 50–130). The mean ATEC score was 50 (range 40–170). There were no significant differences between the experimental and control groups for age, gender, handedness, race, medications, IQ, or ATEC scores.

The experimental group underwent an average of 74 neurofeedback sessions. They were assessed using QEEG, neuropsychological testing, and parent rating scales before treatment and then again after treatment. In order to evaluate the efficacy of neurofeedback treatment for reducing ASD symptomatology, the subjects' scores on the ATEC and neuropsychological testing were compared before and after treatment. A univariate analysis of variance (ANOVA) revealed that ATEC scores changed significantly after treatment ($F = 117.213$; $p < 0.0001$; see Fig. 6.1). Furthermore, 98.8 % of parents reported a reduction in ASD symptoms on the ATEC after treatment.

On objective neuropsychological testing, 100 % of subjects demonstrated some degree of improvement. An ANOVA revealed improvements on tests of visual–perceptual skills ($F=53.6$; $p<0.0001$), language abilities ($F=31.24$; $p<0.0001$), attentional skills ($F=54.04$; $p<0.0001$), and executive functioning ($F=15.65$; $p=0.00015$). In fact, visuoperceptual skills improved 43 %, language abilities improved 47 %, attentional skills improved 56 %, and executive functioning improved 48 %.

Once it was determined that the therapy was efficacious, the next question investigated was whether it had greater efficacy depending on level of functioning or severity of autistic symptoms. We investigated the effects of pretreatment ATEC and IQ scores on treatment outcome by dividing the groups into quartiles based on ATEC and IQ scores and reanalyzing the data. There were no significant differences for any of these analyses. This revealed that (1) ASD symptomatology improved with treatment regardless of IQ and (2) severity of ASD symptoms did not affect treatment outcomes. These results suggest that neurofeedback is an effective treatment regardless of the child's intellectual ability or severity of symptoms, at least within the parameters of the subjects that were included in this study.

6.3.5 Enduring Effects of Neurofeedback on Children with ASD

Both Kouijzer and Coben, along with their respective colleagues, have studied the enduring effects of neurofeedback after the treatment period has ended. One year follow-up data from Kouijzer et al.'s original study demonstrated enduring effects of neurofeedback treatment (Kouijzer et al. 2009a). Repeated measures MANOVA on the executive function task scores at Time2 and Time3 indicated maintenance of cognitive flexibility, planning skills, and verbal inhibition, improvement of attention, and marginally significant improvement of motor inhibition. No significant decreases in executive function skills were found after 1 year. Repeated measures MANOVA comparing Time1 and Time3 data confirmed maintenance of these effects. Analysis revealed significant increases of all executive functions that improved after neurofeedback treatment, i.e., attention skills, cognitive flexibility, inhibition, and planning. Figure 6.2 shows Time1, Time2, and Time3 scores of the treatment group on tests for attention, cognitive flexibility, inhibition, and planning.

Analysis of behavior questionnaires filled out by parents at Time2 and Time3 showed no loss of nonverbal communication and general communication (CCC), social interactions, communication skills, and stereotyped and repetitive behavior (Auti-R). Comparing Time1 and Time3 behavior questionnaires (CCC) confirmed the positive effect for nonverbal communication, but not for general communication. Figure 6.3 shows Time1, Time2, and Time3 questionnaire data (CCC) for general communication and nonverbal communication of the treatment group.

Detailed information about the results of this study can be found in the original paper (Kouijzer, de Moor, Gerrits, Buitelaar et al. 2009).

Analysis of the 6-month follow-up data from their second study (Kouijzer, van Schie, de Moor, Gerrits, and Buitelaar 2009) revealed enduring effects of

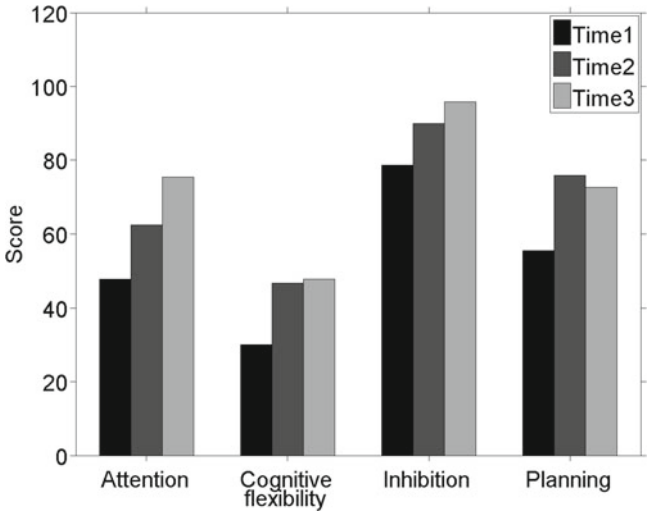

Fig. 6.2 Time1, Time2, and Time3 data of the treatment group on executive function tasks

neurofeedback treatment. Repeated measures MANOVA was used to compare the scores on executive function tasks at Time2 and Time3 and showed no significant changes, suggesting that participants maintained the same levels of executive functioning for at least 6 months. Repeated measures MANOVA comparing Time1 and Time3 data confirmed the previously described effects by revealing a significant increase of cognitive flexibility for the treatment group but not for the control group. Figure 6.4 shows Time1, Time2, and Time3 scores of the treatment and control group on cognitive flexibility.

Repeated measures MANOVA comparing the scores on behavioral questionnaires at Time2 and Time3 showed no effects of group or time, indicating maintenance of the effects in social behavior that were reached 6 months earlier. Repeated measures MANOVA comparing Time1 and Time3 questionnaire data confirmed this effect by showing a significant interaction, suggesting decreases in problem scores on behavior questionnaires for the treatment group, but not for the control group. Figure 6.5 shows Time1, Time2, and Time3 questionnaire data of social interactions and communication skills of treatment and control group.

More detailed information about the results of this study can be found in the original paper (Kouijzer et al. 2009a).

Both studies discussed above indicate maintenance of the effects in executive functions and social behavior from 6 months to 1 year after ending neurofeedback treatment.

A similar study with findings which can be considered complementary to those of Kouijzer and colleagues was recently conducted by Coben at his New York clinic (Coben et al. 2010a). This study assessed 20 patients with ASD in order to investigate long-term clinical effects of neurofeedback in terms of behavioral and

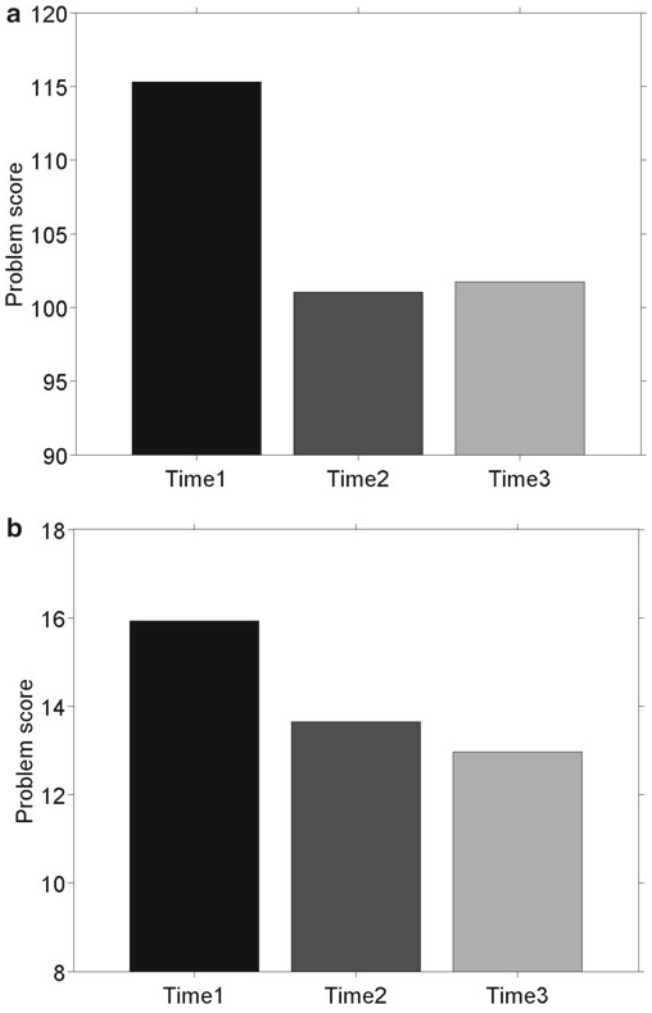

Fig. 6.3 Time1, Time2, and Time3 data of the treatment group on social behavior: general communication (**a**) and nonverbal communication (**b**)

neuropsychological measures. The subject pool for this study was predominately male (16 out of 20 individuals) and all Caucasian. The mean age was 9.53 years, with a range of 5–10 years. Most subjects (80 %) were medication free, with only one subject taking more than two medications. Handedness was mostly right handed ($n = 16$) with one left handed and 3 ambidextrous subjects. Subjects were administered parent rating scales, including the Autism Treatment Evaluation Checklist (ATEC; Rimland and Edelson 2000), the Personality Inventory for Children (PIC-2; Lachar and Gruber 2001), the Behavior Rating Inventory of Executive Function (BRIEF; Gioia, Isquith, Guy, and Kenworthey, 2000), and the Gilliam Asperger's Disorder Scale (GADS; Gilliam 2001). Subjects were also administered

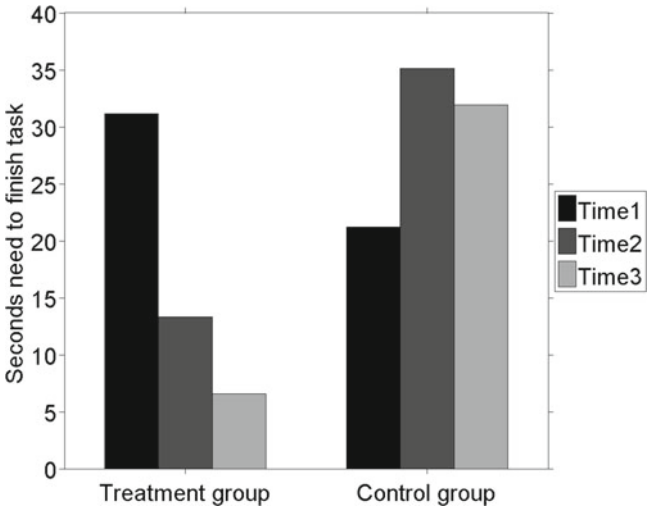

Fig. 6.4 Time1, Time2, and Time3 data of treatment and control group on cognitive flexibility

neuropsychological assessments covering domains of attention/executive functioning, language, and visuospatial processing. After baseline assessments were collected, all subjects underwent at least 40 sessions of neurofeedback training, with an average of 64.5 completed sessions among all subjects. Upon completion of therapy, subjects were reevaluated and pre- and post-treatment scores were compared for significance. After reevaluation, neurofeedback was withheld for between 5 months and 22 months (mean 10.1 months), while no other treatments were administered. Following this break in treatment, subjects were evaluated once again in the same fashion as previously described. Their latter scores were then compared to scores obtained at the end of active neurofeedback training (Time2).

All statistical computations were performed in the statistical package SPSS. Scores prior to treatment on parent rating scales were compared for significance to scores obtained after treatment had ended. Analysis of pre- and postscores obtained from the ATEC revealed significant changes following neurofeedback training. Likewise, changes in scores on the GADS prior to and following treatment were found to be significant. Significant changes were also found to be present following treatment among scores from the BRIEF as well as the PIC-2. Interestingly, when subjects were reassessed following the 5-month to 22-month period of no neurofeedback training, no significant changes were found on any parent rating scale administered (Fig. 6.6). This suggests that changes in parent ratings that were improved by neurofeedback training remained stable during this follow-up period.

Neuropsychological evaluations encompassing the domains of attention, executive functioning, language, and visuospatial processing were also analyzed for significant differences. Significant changes from pre- to post-treatment scores were found among all three domains assessed: attention/executive functioning, language, and visuospatial processing. Interestingly, significant therapeutic changes were also

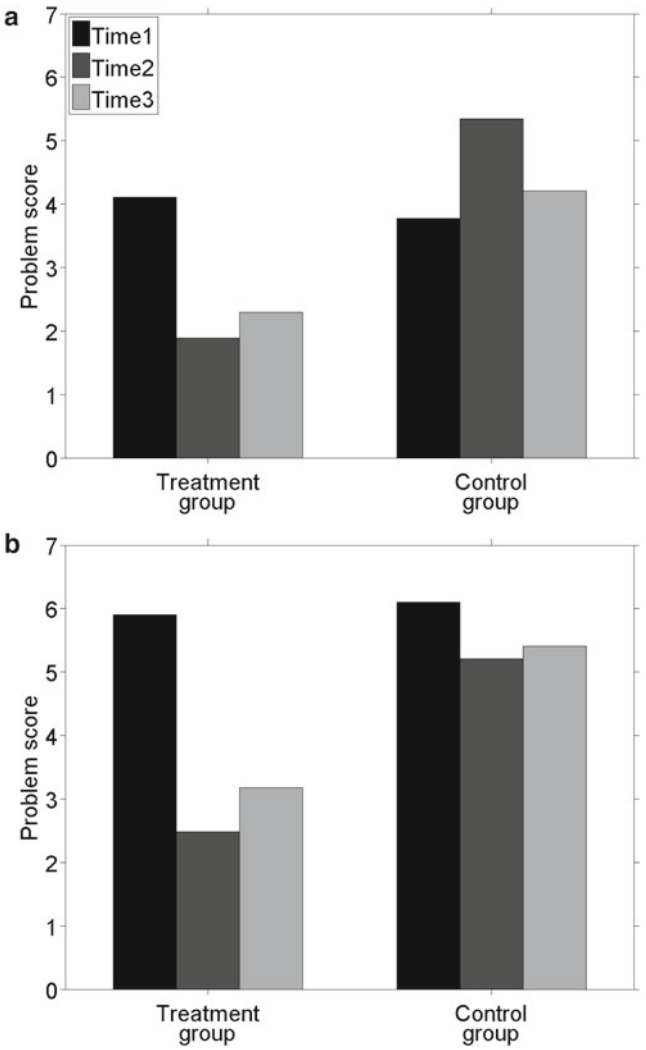

Fig. 6.5 Time1, Time2, and Time3 data of treatment and control group on social behavior: social interactions (**a**) and communication skills (**b**)

found after subjects were reevaluated after a lengthy (5–22 months) absence from neurofeedback training. These occurred in the areas of attention, language, and visuospatial processing (Fig. 6.7). This would suggest that neurofeedback training not only led to objective gains in neuropsychological functioning but that these enhancements in functioning continued to improve over the follow-up period when no treatment was being received.

The results of this present study were quite interesting. First, our findings add to the wealth of studies that have shown that from pre- to posttreatment conditions,

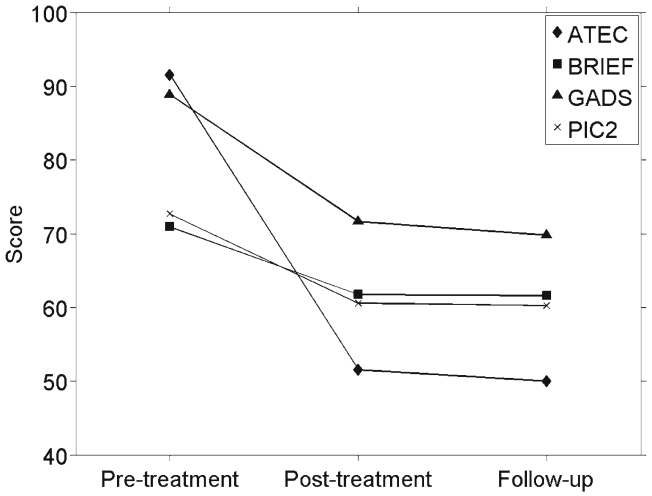

Fig. 6.6 Graph showing the clinical improvements among subjects as assessed by the parents rating scales of ATEC, BRIEF, GADS, and PIC-2 for pretreatment, post-treatment, and follow-up periods

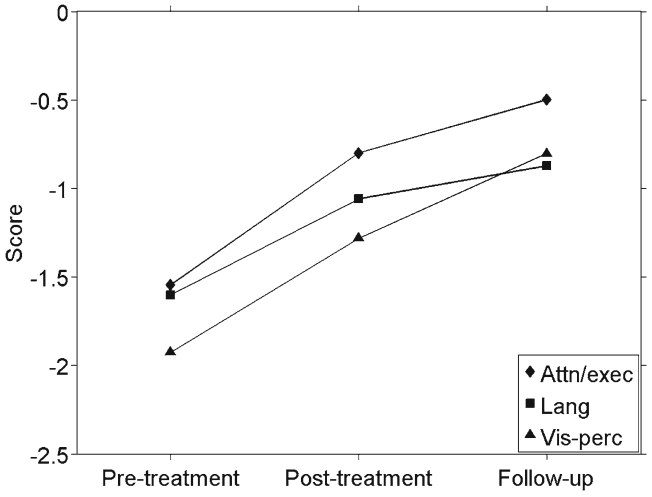

Fig. 6.7 Graph showing the clinical improvements among the domains of attention/executive functioning, language, and visuospatial processing as assessed by neuropsychological evaluations at pretreatment, post-treatment, and follow-up periods

neurofeedback is an effective therapy for treating individuals with autistic spectrum disorders. Additionally, these results show that this treatment was effective in limiting autistic behavioral deficits as well as deficits of a more neuropsychological nature. Furthermore, as our analysis shows, there were no significant increases in autistic pathology when subjects were reevaluated after neurofeedback was

withheld. This finding supports previously found evidence that neurofeedback is capable of creating stable changes within autistic subjects that are not likely to rapidly degrade when treatment ends (Jarusiewicz 2002, p. 749; Coben 2007, p. 740).

Of potentially even greater interest, this study found that during the period in which subjects were receiving no treatment, positive clinical neuropsychological gains were still being manifested within the domains of attention, executive functioning, language, and visuospatial processing. Thus, even without continued treatment, subjects apparently were continuing to improve in these realms. An important implication of this finding is that neurofeedback may indeed change the autistic brain to work in novel and more efficient ways, and these changes may continue to progress even after the treatment has ended. This finding helps further the claim that neurofeedback creates specific neurophysiological changes within the autistic brain (Coben et al. 2009). This is in stark contrast to other commonly administered treatments for autism. For example, Lovaas et al. (1973, p. 1145) performed a study in which applied behavioral analysis (ABA) was administered to a group of children with autism. Upon completion of ABA training, the experimenters reported positive gains in terms of clinical improvements in behavioral deficits. Subjects were then reevaluated between 1 and 4 years later, and subjects who did not continuously receive ABA training had significantly regressed. As our current findings demonstrate, there is no evidence of regression among any of our subjects receiving neurofeedback training. In terms of drug therapies, there is no evidence to our knowledge that would indicate that medications result in enduring clinical gains for subjects with autism when medication is withheld. In fact, numerous studies indicate that prolonged medication use has detrimental effects on autistic individuals (Malone 2002, p. 1149; Anderson et al. 2010).

In terms of the limitations of the current study, the participants consisted of a selected pool of subjects. Subjects were placed in groups by choice of the experimenter rather than by random assignment. When subjects are chosen in that manner, there may be a degree of selection bias associated. We would also recommend that this experiment be replicated with more neuropsychological assessments and parent rating scales included in order to more widely assess the effects of neurofeedback training. This type of investigation could broaden the present findings and help determine if there are other correlations or significant predictors we might not have considered. Also, we would recommend a study with a greater gap between the end of treatment and reevaluation of subjects. Doing this, we believe, would help to assess nature and extent of any positive clinical gains found in subjects when they are no longer receiving treatment, as well as test more fully the limits of enduring effects of neurofeedback treatment.

6.4 Discussion

There are few interventions with proven efficacy for children with autism. Behavioral modification interventions currently have the most empirical support, while pharmacologic interventions, hyperbaric oxygen, and vitamin supplementation have

shown some potential. It is our opinion that neurofeedback is in a similar position with respect to efficacy for ASD, but more research is needed. Neurofeedback is an intervention that may prove to be efficacious in the treatment of symptoms of autism. At present, it should be viewed as possibly efficacious with potential as is the case with most interventions used with this population. Measuring brain-related changes that may occur as a result of neurofeedback is one way of demonstrating its efficacy and mechanism of action. Additional well-designed, more rigorous studies and longer follow-up periods should be included in the future to measure the efficacy of neurofeedback in treating children on the autistic spectrum.

In addition, there is growing evidence that neurofeedback is a therapy capable of creating enduring changes in children with autism. A therapy that can lead to long-lasting effects for children with developmental disorders (and perhaps continuing improvement even after the treatment is stopped) is an enormous asset for children with developmental disorders. Most contemporary treatments require prolonged and lengthy treatment sessions. For example, ABA training can require up to 40 h a week over several months to be effective (Howard 2005, p. 1132). Furthermore, drug therapies usually require years of medication in order to maintain efficacy. In addition, some children require incremental increases in dosages over a period of years for medication use to be clinically viable. Our current results and those of others discussed in this chapter indicate that neurofeedback therapy can reach clinical efficacy relatively quickly and positive gains can be retained for months after treatment has stopped. Outside of the clinical implications, there are ancillary benefits supporting the use neurofeedback. For example, the financial aspects of this treatment should be considered. Presently, the United States alone spends upward of $3.2 million for the care and treatment for a single individual with autism, a figure that equates to $35 billion annually (Ganz 2006).

Results of the studies reviewed in this chapter also provide evidence for the safety of neurofeedback. All studies reported no instances of subjects worsening or showing any side effects while undergoing this treatment over an extended period of time. Moreover, there was no evidence of negative side effects when neurofeedback was ceased. In fact, the opposite was found across all studies. This, again, is contradictory to other interventions, most notably drug therapies, which have documented adverse reactions within this population and often have failed to demonstrate positive effects on primary symptoms (Kidd 2002). Investigations into other contemporary treatments (i.e., diet and chelation therapies) have failed to yield adequate evidence in regard to their safety or efficacy (McDougle et al. 2000; Doja and Roberts 2005; Elder et al. 2006).

We speculate that the enduring effects of neurofeedback in children with developmental disorders are the result of this treatments' ability to change the brain in a therapeutic manner. Recently, Coben and colleagues reported specific neurophysiological changes in terms of coherence within and between specific neural regions following neurofeedback treatment for children with autism spectrum disorder (Coben et al. 2009). We would argue that neurofeedback training causes specific neurophysiological changes within the brain, which in turn contribute to the long-lasting effects of this treatment, and this fosters the continued growth and development of cognitive functions. Moreover, we suggest that more research be conducted

into the precise neural areas clinically affected by neurofeedback in an effort to more fully understand the efficacy of neurofeedback for children with developmental disorders. In summary, results of the studies examined add to the growing wealth of investigations into the efficacy of neurofeedback as a treatment for children with developmental disorders. Moreover, these results have found this treatment to be effective over an extended period of time. Consistent with these results, we recommend future studies be conducted that assess the enduring effects of neurofeedback over even longer treatment spans.

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Biography

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Chapter 7
Structural Imaging in Autism

Brandon A. Zielinski, Molly D. Prigge, Jared A. Nielsen, and Janet E. Lainhart

7.1 Introduction

Advances in magnetic resonance imaging (MRI) have enabled an explosive phase of macroscopic structural brain research in autism. Early studies using this technology characterized gross morphologic features of autistic brains, and detailed differential compartmental volumes of white matter, gray matter, and total brain volume (TBV). More recently, advances in statistical methodology and postprocessing techniques have enabled new insights into relative contributions of regional volumes, thickness of the cortical mantle, surface area, subcortical structures, and telencephalic nuclei to these early findings. Although there are significant discrepancies in this body of work, a consistent picture is beginning to emerge. It now appears that brain regions demonstrating macroscopic structural abnormalities in autism may be different at different ages and undergo differential rates of growth and decline, giving rise to independent trajectories of abnormalities over time. Affected brain substrates may vary by severity, IQ, gender, and developmental stage. Classical concepts of hemispheric dominance, handedness, and lateralization of neuropsychological constructs such as visuospatial processing may not be valid (or consistent) in this disorder. Although current tools have not yet enabled

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researchers to concisely characterize the complexity of this dynamic system, this inhomogeneity itself may represent the very basis of the phenotypic breadth in autism that has dogged scientists for decades.

In this chapter, we first describe the two main techniques used in studies of brain structure in autism: automated volumetry and assessment of cortical thickness. Both of these techniques have added substantially to earlier studies based on gross morphological characterizations of autistic brain structure. In the next section, we review the studies using these methods. We then discuss factors that may influence measures of brain structure in autism. Finally, a glossary of common terms used in structural imaging is presented (Table 7.5).

7.1.1 Automated Volumetry

The technique of using automated methods to measure brain volumes has a long history of iterative improvement. Although it was initially met with some reluctance, automated volumetry has proved to be a robust method for assessing autistic brain abnormalities on the moderate to gross scale, i.e., it is useful for medium and large brain structures. For smaller structures, particularly structures with complex shapes, automated methods often do not work as well; additional steps may be required to ensure measures are accurate. Even for larger structures, steps in the image processing and analysis need to be checked to make sure errors are not being made, particularly in studies of patients whose neuroanatomy may deviate from typically developing individuals. Some of these quality control methods are now automated or semiautomated. Automated volumetry relies on computer-assisted or fully automated delineation of brain structures defined a priori, followed by assessments of characteristic differences in volume, shape, length, position, growth, change, or decline between two study groups. Regions of interest can comprise specific gyri, predefined atlas-based anatomic regions, lobar volumes, hemispheres, or even the whole brain.

7.1.2 Cortical Thickness and Associated Techniques

Similar to automated volumetry, assessment of cortical thickness relies upon computer-assisted delineation of a priori regions of interest. With this technique, the ROI is the cortical mantle. Complex computer algorithms build the cortical sheet using a number of points on the brain surface to distinguish, or "segment," white matter from gray. By digitally excising the cortical surface from surrounding white matter, dura, or CSF, further analyses of the cortical sheet can be performed, including degree of folding, thickness of the gray matter layer, and surface area. Many imaging scientists see this technique as a further level of refinement of volumetric analyses, as cortical volume is the product of thickness × surface area. Divergent

findings result when cortical thickness and surface morphometry are measured. Evidence suggests that they are genetically distinct and reflect differential underlying neurodevelopmental processes.

7.2 Structural Brain Changes in Very Young Children with ASD

We consider structural imaging studies of very young children with ASD at the beginning of our review. These young children are closest in age to the initiating events and the primary pathology of autism. Although the primary pathology is not yet known, changes from typical brain structural development observed in very young children with ASD are strong candidates. Research findings are presented in Table 7.1.

7.2.1 Brain Size and Growth in Early Childhood

Fifteen years ago, the type of abnormalities found in neuropathological studies of children who developed autism suggested that atypical brain development occurred in utero, (Bauman and Kemper 1994). There was concern that autism might be a static disorder in the postnatal period; what went awry in brain development might have happened exclusively before birth, making elucidation of neural mechanisms difficult after birth, particularly after children were diagnosed. Case–control differences in a variety of brain regions had been found in some studies of older individuals with ASD. It was unclear if the changes were developmental defects that originated before birth, or if they had developed later. Research reports of an increased rate of macrocephaly in older children, adolescents, and adults with autism and increased mean TBV in some studies challenged researchers to determine when these differences developed. Because head circumference correlates extremely highly with brain volume during the first few years of life, head circumference data provided the first evidence, albeit indirect, of dynamic changes in brain growth after birth. Macrocephaly in autism was found to usually not be present at birth but to develop during early childhood, inferring an increased rate of early postnatal brain growth (Lainhart et al. 1997). This original finding was quickly replicated by Stevenson et al. (1997) and by other groups (Courchesne et al. 2003; Hazlett et al. 2005) and stimulated neuroscience research in the disorder (Lainhart 2003). The findings provided strong evidence of dynamic changes in brain growth and development after birth in children with autism, and provided hope that in vivo neuroimaging of affected individuals and babies at high risk could help elucidate the neural mechanisms involved.

The period of brain development during the first few years of life is characterized by a dynamic period of rapid expansion of synaptic outgrowth, myelination, and

Table 7.1 Summary of volumetric findings from meta-analyses and recent studies

Reference	Meta-analysis? Datasets (*N*)	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Methods	Behavioral correlations	Frontal lobe
Duerden et al. (2012)	Yes 19	Child: 253 (194 M) Adult: 70 (49 M)	Child: 289 (225 M) Adult: 80 (53 M)	Mean age range Child: 6–15 Adults: 28–38	Child: 159 autism 100 Asperger/ PDD-nos Adult: 31 autism 39 Asperger/ PDD-nos	VBM Volume GM WM	–	Child: ↓ GM bilateral MFG, insula, L IFG, R ACC ↑ GM R SFG, ACC, insula Adult: ↓ GM L IFG, R MFG & SFG, R ACC ↑ GM bilateral MFG, R SFG, L ACC
Ecker et al. (2012)	No	89 M	89 M	26 ± 7 18–43	32 HFA 55 Asperger	VBM Volume GM WM	*ADI-R* – Correlation between occipital–parietal cluster and ADI-R social and communication + Correlation between L GM temporal cluster & ADI-R social No associations with ADOS scores	↑ GM bilateral DLPFC (MFG, precentral)
Mak-Fan et al. (2012)	No	25 M	63 M	10.9 6–15	25 ASD	Volume GM	–	↓ GM with age in ASD only
Nickl-Jockschat et al. (2012)	Yes 16	277	303	Mean age Range: 9–38	–	VBM volume GM WM	–	↓ GM L precentral

Temporal lobe	Parietal lobe	Occipital lobe	Subcortical	Cerebellum	WM regions	Notes
Child: ↓ GM bilateral fusiform, MTG, STG, hippocampus, L ITG, R amygdala ↑ GM bilateral MTG & STG L fusiform Adult: ↓ GM R MTG & STG ↑ GM L hippocampus, R MTG	Child: ↓ GM bilateral precuneus, L IPL & SPL ↑ GM R IPL, precuneus Adult: ↓ GM bilateral cuneus, R precuneus, L PCC	Child: ↓ GM L MOG ↓ GM R MOG, L lingual Adult: ↓ GM R MOG	Child: ↓ GM bilateral thalamus, R globus pallidus, L putamen ↑ GM L putamen, R caudate Adult: ↓ GM bilateral putamen	Child: Regions of bilateral ↓GM & ↑ GM R Adult: Regions of bilateral ↓ GM & ↑ GM	Child: WM bilateral cingulum, corona radiata, R inferior fronto-occipital, L inferior longitudinal & superior longitudinal ↑ WM bilateral corona radiata, L cingulum, extreme capsule Adult: ↓ WM L corona radiata & corticospinal, R inferior fronto occipital & superior longitudinal ↑ WM R corona radiata, L extreme capsule	-
↓ GM R ITG, MTG, fusiform ↑ GM bilateral anterior (ITG, MTG, STG, fusiform, parahippocampus)	↓ GM R PCC, precuneus ↑ GM bilateral postcentral,R IPL	↓ GM bilateral IOG, R SOG, lingual	↑ GM L caudate, putamen, thalamus	↓ GM R	↓ WM volume corticospinal, uncinate, arcuate, inf fronto occipital	–
No differences	↓ GM with age in ASD only	Age-related: ↑ GM in childhood ↓ GM in adolescence ↓ GM with age in ASD only	No differences	No differences	–	–
↓ GM L hippocampus/ amygdala, R MTG	↓ GM L operculum ↑ GM R precuneus Age-related: L pericentral ↓ GM prepuberty, ↑ GM postpuberty in ASD	↑ GM bilateral temporo-occipital, R lingual, IOG	↓ GM bilateral putamen Age-related: R caudate. ↓ GM prepuberty, ↑ GM postpuberty in ASD	↓ GM vermis ↑ GM cerebellum	↓ WM medial anterior cingulate gyrus	–

(continued)

Table 7.1 (continued)

Reference	Meta-analysis? Datasets (*N*)	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Methods	Behavioral correlations	Frontal lobe
Cauda et al. (2011)	Yes 16	350 (290 M)	378 (301 M)	Mean age range: 7–47	66 HFA 86 Asperger	VBM volume GM	–	↓ GM L precentral, R insula ↑ GM R ACC, insula
Hazlett et al. (2011)	No	59 (time 1) and 36 (time 2)	38 and 21	2.7 ± 0.32 and 5.04 ± 0.41	52 autism 7 PDD-NOS (time 1); 33 autism 5 PDD-NOS (time 2)	Volume GM WM	–	No differences
Radua et al. (2011)	Yes 13	Child: 113 Adult: 133	237	Mean age range: 8–37	Child: 80 ASD 18 Asperger Adult: 45 ASD 66 Asperger	VBM Volume WM	–	–
Via et al. (2011)	Yes 20	Child: 272 Adult: 224	Child: 243 Adult: 228	Mean age range Child: 9–17 Adult: 28–38	Child: 189 ASD 47 Asperger Adult: 103 ASD 117 Asperger	VBM Volume GM	No differences in ASD vs. Asperger	↑ GM L MFG, IFG
Yu et al. (2011)	Yes 15	300	404	Mean age range: ASD 9–31 Asperger 11–37	151 ASD 149 Asperger	VBM volume GM	–	ASD vs. TDC: ↑ GM bilateral ACC, R medial, L SFG Asperger vs. TDC: ↓ GM bilateral SFG, medial FG
Jiao et al. (2010)	No	22 (19 M)	16 (13 M)	9.2 ± 2.1; 6–15	ASD	VBM	–	↓ Bilateral medial orbitofrontal, R lateral orbitofrontal, L frontal pole

Temporal lobe	Parietal lobe	Occipital lobe	Subcortical	Cerebellum	WM regions	Notes
↓ GM R amygdala, MTG ↑ GM bilateral MTG, R fusiform	↓ GM bilateral IPL, R precuneus ↑ GM R PCC, precuneus	↑ GM L lingual	↓ GM & ↑ GM R caudate	Regions of ↓ GM and ↑ GM	–	–
↑ WM	No differences	No differences	No differences	No differences	–	
–	–	–	–	–	↓ WM R anterior cingulate, CC ↑ WM R arcuate fasciculus, L inferior fronto-occipital, uncinate	–
↓ GM bilateral amygdala/ hippocampus complex	↓ GM bilateral medial parietal (SPL, postcentral, precuneus)	No differences	No differences	No differences	–	24 Datasets included in the meta-analysis
ASD vs. TDC: ↓ GM L fusiform, R temporal gyrus, hippocampus ↑ GM R parahippocampal, fusiform, MTG, L MTG/ITG Asperger vs. TDC: ↓ GM bilateral hippocampus/ amygdala ↑ GM L fusiform, parahippocampal gyrus/uncus	ASD vs. TDC: ↑ GM R precuneus, postcentral Asperger vs. TDC: ↓ GM R precuneus ↑ GM bilateral IPL	ASD vs. TDC: ↑ GM L lingual Asperger vs. TDC: ↓ GM L occipital	ASD vs. TDC: ↑ GM bilateral caudate Asperger vs. TDC: ↓ GM R putamen	ASD vs. TDC: ↓ GM bilateral ↑ GM R Asperger vs. TDC: ↓ GM R	–	–
↓ Bilateral entorhinal, R temporal pole, L parahippocampal	↓ L caudal ACC	No difference	–	–	–	–

architectural development. Neuronal proliferation and migration and proliferation of radial glial cells are completed in almost all areas of the brain during fetal development. During early postnatal life, there is a dramatic increase in the number of synaptic connections and proliferation of non-radial glial cells (Levitt 2003). The process of myelination rapidly proceeds in a well-organized region-specific manner, and thalamocortical connections continue to come "online" in some regions including the primary auditory cortex (Moore 2002). In typically developing children, brain growth during infancy and early childhood is more important than brain growth during fetal life in predicting IQ at 9 years of age (Gale et al. 2004). Concomitant rapid expansion in essential basic cognitive proficiencies, skill acquisition, motor competencies, language function, and socioemotional processing characterizes this developmental stage. It is also the time when the clinical signs of autism appear.

Interest in dynamic changes in total and regional brain structure in young children with autism was further fueled by early findings suggesting brain overgrowth by age 4–5 years (Courchesne et al. 2001; Carper et al. 2002; Sparks et al. 2002; Hazlett et al. 2005). Studies of TBV in young children at the time of ASD diagnosis, in combination with retrospective head circumference data, have confirmed that the rate of head and brain growth is abnormally rapid during early life in some, but not all children with autism. Hultman et al. (2002), Courchesne et al. (2003), Hazlett et al. (2005), and others confirmed that mean head size (and by inference brain size) is not increased at birth. Courchesne et al. (2003) showed that by 6–14 months of age, a sizeable proportion of children with autism developed macrocephaly. Hazlett et al. (2005) found that mean head circumference in children with autism begins to diverge from mean head circumference of a combined typically developing and non-specific developmentally delayed sample at about 12 months of age. Although the terminology "early brain overgrowth in autism" is frequently used in the literature, it is important to note that the terminology refers to mean brain volume of children with autism considered as a group, i.e., it is a biological tendency in autism.

The majority of young children with autism do not have enlarged heads or brains or abnormally increased rates of brain growth during the first years of life (Lainhart et al. 1997; Lainhart 2003). The proportion of very young children with autism who have abnormally enlarged brains seems to range from 18.6 % [estimated from Fig. 1 in Hazlett et al. (2011)] to 37 % (Courchesne et al. 2001). Some children with autism may have abnormally rapid rates of head and brain growth during the first years of life, but if their heads and brains start out quite small, they may never become macrocephalic or megalencephalic. Abnormal size and rate of growth are two different indicators of potential pathology in brain development in early childhood. There is significant heterogeneity in autism in head and brain size and growth during the first years of life. Although understanding why enlarged heads and brains and increased rates of head and brain growth occur in some very young children with autism, it is equally important to understand why children without these abnormalities develop autism, i.e., it is critically important to understand the variation in these phenomena in the disorder. One study suggests that pattern of autism onset, regressive versus non-regressive onset, may be one influential factor (Nordahl et al. 2011), but confirmation of the results awaits prospective studies using more objective and reliable measures of type of onset.

7.2.2 Global and Lobar Brain and Cerebral Cortex Volumes

Neuroimaging studies of young children have provided information about what parts of the brain seem to be involved in the abnormally increased brain volume when it occurs. Early studies found evidence of increased total brain gray matter and white matter (Courchesne et al. 2001; Hazlett et al. 2005). CSF is not increased but regional group differences are emerging. More recent studies indicate that less global structures, such as gray and white matter in the cerebrum and specific lobes and some subcortical structures, are also increased (Hazlett et al. 2011). Autistic children between 2 and 4 years old have increased mean cerebral gray matter and white matter (Courchesne et al. 2001; Schumann et al. 2010; Hazlett et al. 2011). Within the lobes of the brain, the most consistent results are increased mean frontal and temporal lobe gray matter (Carper et al. 2002; Schumann et al. 2010; Hazlett et al. 2011) and frontal and temporal lobe white matter volumes (Carper et al. 2002; Hazlett et al. 2011). The parietal lobe is less consistently affected. The occipital lobe is usually spared (Carper et al. 2002; Hazlett et al. 2011).

Hazlett et al. (2011) scanned autistic children at approximately 2 years old and again 2 years later. There are several important concepts highlighted by this study. First, widespread "cortical enlargement" was found in temporal, frontal, and parieto-occipital regions, as measured by cortical volume. Cerebral cortical white matter volume was also increased. The differences were present only when cortical gray and white matter volumes were unscaled for TBV. When TBV was used as a covariate, only left temporal lobe white matter was significant (Hazlett et al. 2011). Differences were not found in cortical thickness, leading the authors to infer that surface area was driving the increase volume. These and other authors (Winkler et al. 2010) argue that cortical thickness and cortical surface area derive from distinct neuroembryologic processes and locations and may provide clues to the early cellular or molecular foci of abnormalities. Second, the rate of cerebral enlargement in ASD and controls was parallel across this time span, suggesting that cortical enlargement occurs prior to 2 years of age. In a separate study of young ASD boys 2–5 years of age, Carper and Courchesne (2005) identified enlargement within subregions of the frontal lobe; the dorsolateral prefrontal and medial frontal regions, but not precentral and orbital regions, were enlarged in ASD.

The difference in the Hazlett et al. (2011) results when the volumes were unadjusted versus adjusted for TBV shows that abnormal enlargement is sometimes part of the more global increase in TBV, but other times the enlargement is more spatially specific. Both unscaled and scaled findings may be abnormal, but different mechanisms may be involved—one mechanism global and the other one more local and specific to an individual structure.

Another compelling recent study is that of Schumann et al. (2010), who performed a longitudinal study in autistic children between 2 and 5 years old and normal controls with repeat scans throughout this period. They determined in a relatively robust sample size that autistic children between the ages of 2 and 3.5 years (mean 32 months) had larger total brain (7 %), white matter (10 %), and gray matter (5 %) volumes versus controls (mean 30 months, range 1.6–3.5 years).

Frontal (6 %) and temporal (9 %) gray matter volumes were also larger (lobar white matter volume was not reported). Cingulate volume was increased by 8 %, but the results were not statistically significant. Mixed-effect regression models revealed that all regions except occipital cortex showed increases of volume, or growth trajectory, or both, in autism. Temporal, frontal, and cingulate cortex showed the greatest increases in gray matter volume and growth trajectory.

7.2.3 Subcortical Volumes

7.2.3.1 Amygdala Volume

Four studies examined the amygdala in 2–4-year-old children with ASD. All of the studies found increased amygdala volume bilaterally. Amygdala volume was increased in both unscaled and scaled conditions in two of the studies (Schumann et al. 2009; Nordahl et al. 2012). In the other two studies, mean amygdala volume was significantly increased bilaterally in the unscaled condition but increased only in the right amygdala when TBV was included as a covariate (Sparks et al. 2002; Hazlett et al. 2011). Thus, mean amygdala volume is increased in young children with autism, usually as part of the global increase in TBV. In some cases, there may also be an amygdala-specific effect. Two studies have examined the rate of amygdala growth between 2 and 4 years of age. Mosconi et al. (2009) found no case–control differences in rate of amygdala growth. The amygdala was enlarged in the youngest children but subsequently grew at a typical rate. These results are in contrast with Nordahl et al. (2012) who used a standardized method to correct all T1-weighted images for image distortion that may be caused by MRI scanner hardware variation. Corrected TBVs and amygdala volumes were 12 % and 4.5 % different from the volumes uncorrected for image distortion. With image distortion correction of both TBV and amygdala volumes, they found an increased 1-year rate of amygdala growth in children with autism who were 2–4 years of age at the time of the first scan. Importantly, they considered possible heterogeneity of amygdala growth within the autism group. They found that 42 % of the young children with autism had abnormally rapid rates of amygdala growth relative to TBV growth over a 1-year period, 42 % of the ASD children had normal growth of the amygdala relative to TBV, and 16 % of the ASD group had abnormally slow amygdala growth relative to TBV growth. Growth of the amygdala relative to TBV was also more variable in the ASD children than in the typically developing controls.

7.2.3.2 Hippocampal Volume

The hippocampus may also be abnormal in size in young children with ASD. Sparks et al. (2002) found increased hippocampal volume bilaterally but only when

unscaled for TBV. In a subsequent study by the same research group, Dager et al. (2007) showed that hippocampus shape tended to be abnormal. Saitoh et al. (2001) examined the cross-sectional area of the combined dentate gyrus and CA4 subregion of the hippocampus and found it to be decreased by 13.5 % in young ASD children. In contrast, they found no case–control differences in the combined area of the subiculum and CA1–CA3 subregions of the hippocampus. These results show that when total volume of a structure is increased, subregions of the structure may not be similarly and uniformly affected.

7.2.3.3 Basal Ganglia and Thalamus Volume

Estes et al. (2011) examined the basal ganglia and thalamus in young children with autism. Volume of the right thalamus was increased but only when unscaled for TBV. Volume of the basal ganglia was bilaterally increased but again only in the unscaled condition. Within the basal ganglia, volumes of the left caudate and bilateral putamen, but not the pallidum or right caudate, were increased.

7.2.4 Cerebellar Volume

Volume of the cerebellum has also been examined in young children with ASD. Courchesne et al. (2001) found a 39 % increase in cerebellar white matter, but no case–control differences in cerebellar gray matter volume in 2–3-year-old children with autism. Sparks et al. (2002) found increased mean cerebellar volume but only when it was not corrected for TBV. In contrast, Hazlett et al. (2011) found no case–control differences in total cerebellar volume or cerebellar white or gray matter volumes. A study of the cerebellar vermis volumes found a trend toward decreased volume in the unscaled condition and a significant decrease when volume of the vermis was adjusted for TBV. The finding, although abnormal, was not specific to autism; it was also found in children with non-autistic developmental delay, but different subareas of the vermis were affected.

7.2.5 Corpus Callosum Size

One structural imaging study in young children with autism measured cross-sectional area of the corpus callosum (Boger-Megiddo et al. 2006). CC area was decreased in the young ASD children but only relative to TBV. Widespread areas of the CC were affected. CC area was decreased in non-autistic young children with developmental delay in both unscaled and scaled conditions.

7.2.6 Quantitative T2 Relaxation and Myelin Transfer Imaging

Two studies used non-volumetric types of imaging to get more specific physical information about gray and white matter in autism. In the first study, prolonged T2 relaxation was found in cortical gray matter but not cerebral white matter in children with ASD 2–4 years of age (Petropoulos et al. 2006). Quantitative T2 relaxation measures complex brain factors affected mainly by myelin and intracellular and extracellular brain water. In the brain of a young infant, brain water is increased and myelin is at a minimum, resulting in less constraint on T2 relaxation, which is slower (more prolonged) at this young age compared to older children. As the typical brain undergoes early maturation during the first few years of life, decreasing brain water and increasing myelination result in increased compactness of brain tissue, and the increased tissue compactness makes T2 relaxation faster. The prolonged T2 in young children with autism suggests that the increased rate of brain growth does not represent advanced "normal" brain development or maturation. Rather, the prolonged T2 relaxation suggests that the tendency toward an increased rate of brain growth, which results in an abnormally large brain in some young ASD children, represents a state of whole-brain cortical gray matter underdevelopment, likely due to delayed neuronal structural growth. The results suggest a pathologic process in gray matter development in young children with autism. The authors point out the possibility that white matter T2 relaxation could be affected later in childhood, in a more progressive manner. In contrast to the ASD group, non-autistic young children with developmental delay had prolonged T2 in both gray matter and white matter, suggesting delayed neuronal development and myelination.

The second investigation examined physical aspects of brain tissue with magnetization transfer imaging (MTI). The corpus callosum was studied with MTI in 101 young children with autism and 35 typically developing children (mean ages 4.25 years) (Gozzi et al. 2012). MTI is an indirect measure of myelination. It is sensitive to age-related changes in myelination, which is normally a tightly regulated process. MTI measures different aspects of myelination than T2 relaxation. The magnetization transfer ratio (MTR) is independent of fractional anisotropy measured by diffusion tensor imaging. In contrast to the quantitative T2 relaxation study described above that did not detect evidence of abnormal myelination in cerebral white matter in young children with autism, the MTI study found evidence of altered myelination in the corpus callosum. In the young children with ASD, mean MTR was increased compared to the typically developing sample. The investigators carefully and systematically considered a number of potential confounding factors, but none of the factors explained or significantly changed the results. The results suggest that at this very young age, the process of myelination in the corpus callosum is abnormal. Combination of MTI with other types of in vivo imaging and postmortem and animal studies will be necessary to determine the type of abnormality in myelination that is present.

7.2.7 Male–Female Differences

Gender differences were reported in a number of the studies of total and regional volumes and rates of growth during early childhood in ASD (Sparks et al. 2002; Schumann et al. 2009, 2010; Nordahl et al. 2011). For example, boys with autism showed differences in TBV, but not total gray or white matter, frontal and temporal gray matter, and growth trajectory in cingulate cortex relative to controls. Females, in contrast, showed increases in total brain, gray and white matter volumes, and cingulate gray, as well as total brain, total gray, frontal and temporal gray growth trajectories versus controls. No direct autistic male versus autistic female comparisons were reported.

7.2.8 Summary

In summary, the studies in young children with autism suggest that abnormal brain growth occurs even earlier than age 2.5 years in autism and in some cases may continue through early childhood, at least in some subregions. The results demonstrate that different abnormalities may occur in structure size and rate of growth, when considered in absolute terms and relative to TBV. The results underscore that male–female differences in brain volume and growth trajectory are an important consideration in this young age group. The results provide evidence of heterogeneity of brain volumes and rate of brain growth in young children with autism. They suggest the importance of understanding this variation and testing for neurobiological subtypes of autism defined by brain volume and rate of growth.

In an effort to extend these advances to even younger children with autism, the Infant Brain Imaging Study (IBIS) Network is collecting data from infants at high familial risk for developing autism. These infants have at least one older sibling with the disorder. An initial report of brain volumes at 6 months of age (Hazlett et al. 2012) detected no differences in head circumference or brain volume in the high-risk infants versus infants with low familial risk for autism. As these children grow older, some of them may ultimately develop clinical features of autism, enabling reanalysis of these data by diagnosis. This could become a very rich dataset but will greatly depend on how many of these children develop the disorder. Some research groups are now studying babies at high risk for autism beginning at an even younger age (Table 7.2).

Table 7.2 Results of structural imaging studies in young children with ASD

Imaging research team leader	Eric Courchesne Ph.D.	Steven Dager M.D.	Joseph Piven M.D.	David Amaral Ph.D.
Age range	22–67 months	2–4 years	18–35 months at baseline scan and follow-up after 2 years and 6 months in high risk study	25.6–47.0 months at baseline scan and follow-up after 1 year
Representative studies: groups compared and sample sizes	Schumann et al. (2009) $n=41$ ASD (32 M, 9 F) $n=44$ TD (32 M, 12 F) Saitoh et al. (2001) $n=11$ ASD $n=9$ ASD Courchesne et al. (2001) $n=30$ ASD $n=12$ TD	Sparks et al. (2002), Boger-Megiddo et al. (2006), Webb et al. (2009), Estes et al. (2011) $n=45$ ASD (38 M, 7 F) $n=26$ TD (18 M, 8 F) $n=14$ DD (6 M, 8 F) Petropoulos et al. (2006) $n=60$ ASD $n=16$ DD $n=10$ TD	Mosconi et al. (2009) $n=50$ ASD $n=22$ TD $n=11$ DD Hazlett et al. (2011) $n=59$ ASD $n=26$ TD $n=12$ DD Hazlett et al. (2012) $n=98$ high risk $n=36$ low risk	Nordahl et al. (2011) $n=53$ ASD-nREG[a] $n=61$ ASD-REG $n=66$ TD Nordahl et al. (2012) $n=85$ ASD (all M) $n=47$ TD (all M)
Tesla	1.5 T	1.5 T	1.5 T and 3 T	3 T
Results				
Cerebrum	Increased[b] frontal and temporal lobe gray matter and difference in rate of growth for gray matter of all lobes Increased whole brain, total white matter, and total gray matter	Increased -sp[c]	Increased in all lobes at both scans (only total temporal lobe and temporal lobe white matter survive scaled-TBV), but no difference in rate of growth -sp No difference[d] in high risk	Increased: ASD-REG No difference: ASD-nREG
–	–	*Unscaled*[e]	*Scaled-TBV*[f]	–

Cerebellum	Increased in white matter No difference in gray matter	Increased -sp Trend decreased in vermis -nsp[g]	No difference Decreased in vermis—nsp	No difference at 2 year or 4 year in ASD or 6 months in high risk	–
Hippocampus	Decreased in area dentata	Increased -sp and abnormal shape	No difference	–	–
Amygdala	–	Increased -sp	No difference (except in boys' right hemisphere)	Increased at both scans, but no difference in rate of growth (only right hemisphere effect survives scaling-TBV)	Increased at both scans and increased rate of growth (scaled-TBV)
Corpus callosum	–	No difference	Decreased -nsp	–	–
Thalamus	–	Increased in right hemisphere -sp	No difference	–	–
Basal ganglia	–	Increased in striatum, left caudate, right caudate (trend), and putamen -sp No difference in pallidum	No difference	–	–
T2 cortical relaxation	–	Prolonged in cortical gray matter in ASD and DD; no difference in white matter in ASD, prolonged DD		–	–
Head circumference	–	–		No difference at 6 months	Increased by 4–6 months: ASD-REG No difference: ASD-nREG

[a]ASD-nREG = ASD without regression, ASD-REG = ASD with regression
[b]Increased = significantly increased in ASD vs. TD comparison
[c]Sp = Case-control difference was specific to ASD vs. typical development (TD), not found in Nonspecific developmental delay vs. TD comparison
[d]No difference = not significantly different in ASD vs. TD comparison
[e]Unscaled = without TBV as a covariate
[f]Scaled-TBV = with TBV as a covariate
[g]Nsp = Case-control difference was not specific to ASD, it was found in both ASD vs. TD and DD vs. TD comparison

7.3 Global Brain Changes in Older Children, Adolescents, and Adults with ASD

After the first 2 years of life, the average autism brain enters a phase of plateaued growth. When viewed against the backdrop of persistent growth in normal children, this phase can be interpreted as a relative decline in the growth rate *below* normal. Around mid-childhood (5–10 years of age), the average autism brain is no longer much larger than normal controls (Herbert et al. 2003; Kates et al. 2004). Both white matter and gray matter exhibit markedly slowed rates of growth (Courchesne et al. 2001). A preliminary longitudinal study of 13 boys with autism and 7 typically developing boys, each scanned twice 3 years apart, found whole brain white matter to be growing robustly in the typical boys but to be slowed in the boys with autism (Hua et al. 2011). By late childhood, adolescence, or young adulthood, mean brain volumes in autism and normal controls samples do not differ, even though mean head circumference and rates of macrocephaly remain increased in the autism groups (Courchesne et al. 2001; Hardan et al. 2001; Aylward et al. 2002). This concept is further supported by the work of Courchesne et al. (2011), who investigated 259 autistic subjects aged 2–50 years and 327 controls across a similar age range. Their mixed cross-sectional and longitudinal data provide additional evidence that autistic TBV is already enlarged versus controls at the age of 2–3 years and that growth trajectory increases through early childhood, plateaus in later childhood reaching an inflection point around age 8–9 years (though somewhat earlier for females), and shows accelerated decline beyond early adulthood. The results converge to show that the trajectory of early brain overgrowth, followed by plateau and then late decline, is a consistent trend in the disorder (Courchesne et al. 2004, 2011). Nevertheless, there is a paucity of data in much of childhood, most of the data come from predominantly cross-sectional rather than longitudinal MRI studies, and specific regional abnormalities remain understudied.

The apparent normalization of TBV between early childhood and young adulthood may represent pseudo-normalization; the processes occurring in the brain may not be normal late brain development and maturation. In typically developing individuals, dynamic changes in circuitry and regional and subregional brain morphometry occur during later childhood, adolescence, and young adulthood despite little change in TBV (Caviness et al. 1996; Sowell et al. 2002; Giedd 2004). These changes may reflect the emergence of consolidated large-scale brain networks (Zielinski et al. 2010). Brain development in normal adolescents sets the stage for mature cognitive functioning.

In contrast to typical development, adolescence and young adulthood in autism are often times when cognitive and overall functioning plateau or deteriorate and seizures may develop. Individuals with autism who do not deteriorate during adolescence and young adulthood for the most part remain significantly impaired. Therefore, the brain changes associated with "normalization" of brain volume in autism are likely inherently pathologic or a complex mixture of pathology, compensatory mechanisms, relatively normal processes, and silent "sculpting" of the brain by the often atypical life experiences of individuals with autism.

7.4 Regional Abnormalities in Older Children, Adolescents, and Adults with ASD

7.4.1 The Cerebral Cortex

In this section of the chapter, the cerebral cortex, the thin strip of critical gray matter on the surface of the brain, is the region of interest. Although no differences in cortical thickness have been found in very young children with ASD (Hazlett et al. 2011), differences have been observed in older individuals. We compare the results from the very young and older individuals with ASD with caution. The young and older ASD samples differ in several important ways in addition to the stage of brain development. The very young ASD sample (Hazlett et al. 2011) included mostly boys (86 %) but also examined girls. In contrast, over half of the studies of older individuals focused exclusively on males to decrease heterogeneity. The young sample included cognitively high-functioning and low-functioning children with ASD. All studies of cortical thickness in older individuals have been performed in cognitively high-functioning ASD individuals (full-scale IQ ≥ 70, often average and above-average IQ), once again to decrease heterogeneity in the ASD sample, which may be greater at older than at younger ages.

Two basic types of abnormalities are described. The first type of abnormality is a difference in cortical thickness at any age, i.e., either thicker or thinner in ASD (Chung et al. 2005; Hadjikhani et al. 2006; Hardan et al. 2006; Dziobek et al. 2010; Hyde et al. 2010; Wallace et al. 2010; Scheel et al. 2011; Misaki et al. 2012). The second type of abnormality is a difference in age-related change in cortical thickness, i.e., age × diagnosis interactions (Chung et al. 2005; Hardan et al. 2009a; Raznahan et al. 2010; Wallace et al. 2010; Scheel et al. 2011; Mak-Fan et al. 2012; Misaki et al. 2012). The age-related changes in cortical thickness in ASD reported to date must be considered preliminary because they are predominantly from cross-sectional data, which can only infer developmental trends from changes across individuals (Kraemer et al. 2000). The findings need to be confirmed in longitudinal studies that directly measure development, i.e., change with age within individuals.

The combined results of studies of cortical thickness in individuals with ASD 2–60 years of age suggest dynamic cortical dysmaturation. The results suggest several important stages and patterns of dysmaturation (Table 7.3). First, the lack of any difference in cortical thickness in very young children with ASD and increased cortical thickness in older children suggests that cortical thickness increases at an abnormally rapid rate between very early and late childhood. The second major transition in cortical thickness in ASD appears to occur in adolescence. Cortical thickness in adolescence in ASD is thinner than typical in some areas but no different than typical in other areas. At this stage of development, age-related trajectories of cortical thickness in typically developing samples and ASD samples cross or begin to merge. The cortex is thinning more than typical with age in some areas in ASD. In other areas, the cortex is not thinning as much (or at all) as expected.

Table 7.3 Differences in cortical thickness in ASD compared to typical development

Age	2–4 years	4–8 years[a]	8–12 years	Adolescence	Adulthood
Summary of study results	No difference	? Transition[a] to thicker	Thicker	Transition to thinner in some regions	Thinner in some regions; no different in other regions
Areas of the cortex most affected			Temporal	Frontal, temporal, parietal	Frontal, temporal, parietal

[a]Data are limited in this age group

Several excellent studies show the trend between older childhood and adulthood in cortical thickness in ASD. Raznahan et al. (2010) is an illustrative example. These investigators studied a wide age range of individuals with autism and control subjects (10–60 years) to clarify differential contributions to structural changes in thickness, volume, and surface area. Using a predefined parcellation scheme, they report age by diagnosis interactions of volume and thickness, but not surface area in fusiform and middle temporal gyrus. In these regions, there was an accelerated decrease in volume and thickness in ASD. Using a parallel "shotgun" approach, analyzing multiple brain surface points, widespread (but focal) interactions with age in ASD were found in temporal, parietal, and frontal cortices. All of these regions demonstrated decreased volume and thickness in young ASD subjects that remained stable through adulthood but continued to normally decline in control subjects. The result led to a tendency toward no difference from controls (or at times increased thickness) in older adults with ASD. Two important methodological conclusions may be drawn from this work. First, studies that utilize gross anatomic parcellation schemes (i.e., large-volume averaging) may have insufficient sensitivity to detect structural brain changes. Second, cortical dysmaturation in ASD may extend throughout adulthood and in some regions may be progressive. The functional significance of these changes is uncertain at this time.

As suggested by the Raznahan et al. (2010) study and other studies, cortical thickness abnormalities in ASD are region-specific. In addition, as mentioned above, age-related abnormalities in cortical thickness are of two different types. The first type is a region-specific *lack* of normal cortical thinning in adolescence and adulthood, i.e., lack of thinning in areas that are thinning in typical development. The second type of age-related cortical disturbance is region-specific *abnormal* cortical thinning, i.e., thinning in areas of the cortex that are not decreasing in thickness in typically developing individuals. Areas of the frontal lobe with abnormal thinness or atypical trajectory in ASD include superior frontal, inferior frontal, medial frontal, left prefrontal, right orbitofrontal, right precentral, and right paracentral regions. In the temporal lobe, the banks of the left superior temporal sulcus are repeatedly implicated along with the left fusiform and inferior temporal gyrus. Other areas of the temporal lobe affected in some studies include the left entorhinal, middle temporal, and parahippocampal regions. In the parietal lobe, the superior, inferior, and medial parietal and supramarginal, postcentral, and cuneus areas are involved.

Very interestingly, the occipital cortex is usually spared. Six of seven studies find no abnormalities of thickness in occipital cortex, and only one of six studies examining age-related changes in cortical thickness find atypical trajectories in occipital cortex. The one positive study, which is noteworthy because it is the first and currently the only longitudinal study of cortical thickness in autism, examined age-related changes over a short (2.5 years) period (Hardan et al. 2009a). The studies of cortical thickness and other cortical features in ASD published to date are summarized in Table 7.4.

Longitudinal MRI studies of cortical thickness across the life span in autism are needed to confirm results found to date and to clarify structural changes in cortical development in the context of cognitive function during this final critical period of brain development. In a preliminary longitudinal analysis, Hardan et al. (2009a) described changes in thickness and volume between subjects aged 8–12 years at baseline, who were subsequently rescanned approximately 2 years later. The ASD group tended to have accelerated decreases in GM volume and cortical thickness in the temporal and occipital lobe. Decreases in occipital cortical thickness were most robust. Greater thinning was associated with greater symptom severity on social scales (frontal) and motor stereotypies (temporal lobe). Notably, subjects were not matched on IQ, and intergroup differences did not withstand correction for IQ.

More fine-grained regional assessment of cortical thickness and advanced multivariate, including canonical correlational analysis, methods [exemplified by Misaki et al. (2012)] will provide much insight into compartmental changes across age related to autism. Beyond mid-adulthood, imaging data regarding the aging brain in autism are lacking. Understanding aging in autism will become increasingly important as the disorder is more frequently recognized and diagnosed in older individuals and as therapeutic interventions begin to extend the functional lives of autistic patients. How abnormal changes in cortical thickness are related to abnormal changes in other parts of the brain need to be determined. Finally, the causes of abnormal cortical thickness development and maturation in ASD need to be determined. The studies above provide evidence of cortical decline extending through adolescence and into adulthood in some individuals with the disorder.

7.4.2 Subcortical Structures

7.4.2.1 Amygdala

Is increased mean amygdala volume, consistently found in the studies of very young children with autism, observed in studies of older children, adolescents, and adults with ASD? The answer to this question is "no," with the caveat that the answer is inferred from changes across individuals in cross-sectional studies rather than from longitudinal data. A meta-analysis of six studies found insignificant effect sizes (ES = 0.15 left amygdala and 0.28 right amygdala) and no evidence of age-related heterogeneity (Stanfield et al. 2008). Some more recent studies, such as

Table 7.4 Summary of studies reporting cortical thickness and surface area in autism, organized chronologically then alphabetically

Reference	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Methods	Behavioral correlations	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe	Notes
Mak-Fan et al. (2012)	25 M	63 M	10.9 6–15	25 ASD	Cortical thickness, surface area	–	*Thickness* ↓ With age in ASD only ($p = 0.054$) ↑ L IFG (at 7.5 year)	No difference	*Thickness* ↓ With age in ASD only ↑ L medial parieto-occipital/ precuneus (at 7.5 year)	*Surface area* ↑ In ASD at older age range	–
Misaki et al. (2012)	41	40	16.8 ± 2.8; 12–24	11 HFA 26 Asperger 3 PDD-NOS	Cortical thickness	*FSIQ* + correlation with bilateral postcentral and R STG in TDC; – correlations in ASD	*Thickness* ↓ L middle frontal Age-related: L middle frontal ↓ age in TDC only	*Thickness* Age-related: L parahip-pocampal ↓ age in autism only	*Thickness* ↓ L superior parietal, R paracentral Age-related: R superior parietal ↓ age in TDC only	No difference	Reanalysis of data from Wallace et al. (2010)
Shokouhi et al. (2012)	15 M	16 M	15.4 ± 2.2 12–20	ASD	Cortical sulcal index, surface, length, depth	–	*Sulcal surface* ↑ L insula	No difference	*Sulcal length* ↑ R intraparietal	No difference	–
Hazlett et al. (2011)	59 (time 1) and 36 (time 2)	38 and 21	2.7 ± 0.32 and 5.04 +/– 0.41	52 autism 7 PDD-NOS (time 1); 33 autism 5 PDD-NOS (time 2)	Cortical thickness, surface area	–	↑ Surface area	↑ Surface area	↑ Surface area parieto-occipital	↑ Surface area parieto-occipital	Surface area estimated from regional cortical volume/ thickness

Scheel et al. (2011)	28 (18 M)	28 (18 M)	33.1 ± 9.7; 20–55	HFA	Cortical thickness (three approaches: direct comparison (DC); DOSS—different offsets, same slope; and DODS—different offsets, different slope)	–	DOSS: ↓ R precentral gyrus, paracentral lobule DODS: ↓ R precentral gyrus Age-related: R precentral ↓ with age in TDC only	DC & DOSS: ↓ L posterior superior temporal sulcus, middle temporal gyrus	DC & DOSS: ↓ L supramarginal gyrus DODS: ↓ L intraparietal sulcus, inferior parietal, R postcentral gyrus, and bilateral supramarginal gyrus Age-related: R postcentral, L intraparietal sulcus & supramarginal ↓ with age in TDC only	DOSS: ↓ L cuneus, lingual gyrus	–
Dziobek et al. (2010)	27 (20 M)	29 (22 M)	42 ± 11.3	9 HFA 18 Asperger	Cortical thickness, amygdala volume, and fcMRI	ASD: negative relationship between fusiform thickness and face recognition	No difference	*Thickness* ↑ L fusiform gyrus L fusiform thickness + correlation with amygdala volume in TDC only	No difference	No difference	–

(continued)

Table 7.4 (continued)

Reference	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Methods	Behavioral correlations	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe	Notes
Hyde et al. (2010)	15 M	15 M	22.7 ± 6.4 (14–33)	HFA	Cortical thickness	–	↑ Bilateral medial, middle, medial orbital frontal gyri ↑ R anterior cingulate gyrus, inferior and superior frontal gyri ↓ R precentral gyrus	↑ Bilateral fusiform gyrus, superior temporal sulcus, Heschl's gyrus	↑ Bilateral posterior cingulate gyrus ↑ R inferior parietal lobule ↓ R post- and paracentral gyrus	↑ L lingual gyrus, ↑ R middle occipital gyrus	–
Jiao et al. (2010)	22 (19 M)	16 (13 M)	9.2 ± 2.1; 6–15	ASD	Classification using 4 machine-learning algorithms comparing accuracy of cortical thickness vs. VBM features	–	*Thickness* ↓ Bilateral lateral orbitofrontal, pars triangularis*, L medial orbitofrontal*, frontal pole*, R medial orbitofrontal, rostral middle frontal ↑ L caudal anterior cingulate*	*Thickness* ↓ Bilateral entorhinal temporal pole, L parahippo-campal*, R parahippo-campal	Thickness ↑ L precuneus*, superior parietal	No difference	Logistic model trees performed best for both cortical thickness (87 % accuracy) and volumetric (74 % accuracy) features. Seven most informative regions * in previous columns

Raznahan et al. (2010)	76 M	51 M	31.7 ± 12.1; 10–60	10 Autism 62 Asperger 4 PDD-NOS	Cortical thickness, surface area	–	*Thickness* Similar age-related ↓ to TDC	*Thickness* Age-related ↓ middle temporal, fusiform found in TDC, not ASD	*Thickness* Similar age-related ↓ to TDC	*Thickness* Similar age-related ↓ to TDC	–
Wallace et al. (2010)	41 M IQ < 70	40 M	12–24 year	11 HFA 26 Asperger 3 PDD-nos 1 Asp or HFA	Cortical thickness	–	No difference	↓ L inferior temporal, entorhinal, fusiform, banks superior Age-related ↑ thinning in left fusiform/ inferior temporal cortex	↓ Bilateral superior and inferior, L supramarginal and postcentral	No differences	Data was reanalyzed in Misaki et al. (2012)
Hardan et al. (2009a)	18 M	16 M	2 Scans: 10.9 ± 1.2 13.3 ± 1.4	All autism	Cortical thickness at 2 time points (separated by 30 months)	ADI-R—correlation between socioemotional reciprocity and change in frontal cortical thickness	No difference	*Thickness* ↓ in ASD (did not survive multiple comparison correction)	No difference	Greater ↓ in cortical thickness in ASD	–
Hutsler et al. (2007)	8	8	27.6 15–45	7 autism 1 Asperger	Postmortem MRI cortical thickness (and histology)	–	No difference	*Thickness* ↑ In autism (prior to controlling for multiple comparison)	No difference	No difference	–

(continued)

Table 7.4 (continued)

Reference	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Methods	Behavioral correlations	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe	Notes
Hardan et al. (2006)	17 M	14 M	10.5 ± 1.5 8–12	Autism	Cortical thickness, gyral & sulcal thickness	–	No difference	↑ Total lobe, gyral, sulcal thickness	↑ Total lobe thickness	No difference	–
Hadjikhani et al. (2006)	14 M	14 M	33 ± 12	8 HFA 4 Asperger 1 PDD-NOS	Cortical thickness	ADI-R Social + Communication score correlated with thinning: Inferior frontal gyrus pars opercularis, inferior parietal lobule, R superior temporal sulcus	↓ Inferior frontal, precentral gyrus (motor face area), orbitofrontal cortex, prefrontal cortex, anterior cingulate	↓ Superior temporal sulcus, inferior and middle temporal gyrus	↓ Inferior and superior parietal lobules, postcentral gyrus (sensory face area), supramarginal gyrus, medial parietal cortex	↓ Inferior and middle occipital gyrus	–
Chung et al. (2005)	16 M	12 M	16.1 ± 4.5; 10–25	16 HFA	Cortical thickness via heat kernel smoothing	–	↓ R inferior orbital prefrontal	↓ L superior temporal sulcus	No difference	↓ L occipito-temporal gyrus	–

*Defined in the 'notes'; right–most column for this paper

O'Brien et al. (2010), found no case–control differences in amygdala volume. Numerous other studies have examined amygdala volume in ASD. For the purpose of this review, we describe several illustrative examples.

Schumann et al. (2004) compared ASD-control differences in participants divided into 2 age bins: 7.5–12.5 years and 12.75–18.5 years. Left and right amygdalae were significantly larger in the younger autism group, but not the older autism group compared to typically developing controls, despite no differences in total cerebral volume. Because the amygdala was substantially growing in volume in later childhood and adolescence in the typically developing controls, the findings in the autism groups suggested that the amygdala was not growing as expected during this period in autism (Schumann et al. 2004). This cross-sectional finding has been replicated in a larger study of a wider age range of individuals with ASD. Murphy et al. (2012) examined amygdala volume in 32 young people with Asperger syndrome and 32 typically developing controls ranging in age from 12 to 47 years of age (Murphy et al. 2012). Although there were no case–control differences in TBV, they examined amygdala volume uncorrected and then corrected for TBV (as mentioned above, if an abnormality in volume is detected, examining case–control differences in both the TBV uncorrected and corrected conditions provides information about whether the abnormality is part of a more global brain disturbance or is more specific to the amygdala). Mean total, left, and right amygdala volumes were increased in the autism group without correction for TBV. When TBV was included as a covariate, total and right amygdala volumes remained significantly increased in the Asperger syndrome group. Note that this finding is similar to what was found in two of the four studies of the very young children with autism discussed above; case–control differences only in the right amygdala remained significant when TBV was included as a covariate (Sparks et al. 2002; Hazlett et al. 2011). Testing for an age × diagnosis interaction, Murphy et al. (2012) found that volume of the amygdala was increasing in the typically developing group well into adulthood, but there was *no* apparent growth in the Asperger syndrome group. These results need to be confirmed by longitudinal studies. The combined results suggest dynamic dysregulation of amygdala growth from very early childhood into mid-adulthood.

Is there evidence of a subgroup effect based on amygdala abnormalities in the older ASD individuals as was found in the very young ASD children? Little research has been done to test for significant neurobiological subtypes of ASD based on amygdala size in older individuals with ASD. The investigation by Salmond et al. (2003) remains the most informative study. They used voxel-based methods adapted to detect gray matter deficits in individual subjects with ASD. Each ASD individual was compared to the entire control group. They constrained their analysis to bilateral abnormalities only. Fourteen children with autism, ranging in age from 8 to 18 years (mean age 12.9 years), were compared to 14 age-matched typically developing children. Although the findings must be considered preliminary because of the small sample sizes, the results are noteworthy. Seven of the 14 (50 %) children with autism were found to have bilateral defects of gray matter density in the amygdala. The other seven autism children had no amygdala volume abnormality detected by

this method. Age effects were not examined nor was whole brain gray matter tested as a covariate. Age × diagnosis interactions and TBV effects on amygdala volume are important to consider in future studies.

Do volumetric changes in the amygdala have functional consequences? At the present time, only correlates of amygdala volume and cross-sectional predictors of variability, rather than potential consequences, have been examined. Increased right amygdala volume was significantly correlated with increased severity of clinical social and communication scores in young children with ASD (Munson et al. 2006). In older individuals with ASD, 8–25 years of age, smaller amygdala volume was associated with less fixation on eye regions in a facial expression judgment task, and slowness in distinguishing facial expressions (Nacewicz et al. 2006).

7.4.2.2 Hippocampus

In very young children with autism, hippocampal volume was increased bilaterally but only when unadjusted for TBV (Sparks et al. 2002). The findings suggest that hippocampal volume is indeed abnormal in young children with autism, but the abnormality appears to be part of the more global increase in TBV seen at this age, rather than specific to hippocampal development.

In older children, adolescents, and adults with ASD, the results of studies examining hippocampal volume are quite mixed. Schumann et al. (2004) found evidence of mean hippocampal enlargement in older children and adolescents with autism, and case–control differences in right hippocampal volume remained significant after adjusting for TBV (Schumann et al. 2004). A meta-analysis of six studies, including Schumann et al. (2004) failed to find significant effect sizes for case–control differences in hippocampal volume (ES = 0.41 left hippocampus and 0.29 right hippocampus) or significant effects of age or IQ (Stanfield et al. 2008). More recently, hippocampal volume was not found to significantly differ in individuals with ASD 12–47 years of age, and cross-sectional age-related changes in hippocampal volume were similar in ASD and controls (Murphy et al. 2012). Abnormalities of hippocampal shape were found in very young children with autism (Dager et al. 2007). Shape differences have also been found in older individuals with the disorder (Nicolson et al. 2006). The functional consequences of hippocampal shape differences in autism are not yet known.

Some of the inconsistency in studies of hippocampal volume may be due to different rates of macrocephaly in the autism samples studied, lack of control for brain volume, and possible developmental effects on case–control differences and on the scaling of the hippocampus and TBV. Individuals with autism and macrocephaly have larger hippocampi and amygdalae than normocephalic autism subjects (Bigler et al. 2003). It is also plausible that the lack of consistent results across studies may reflect lack of consistent morphometric abnormality; the proportion of individuals with hippocampal abnormalities may differ in different samples. Using sensitive methods to detect individual differences, Salmond et al. (2005) found bilateral gray matter deficits in the hippocampus in 50 % of children with autism. In addition, the

possibility of dynamic local increases and decreases in parts of these structures with little or no change in overall volume needs to be investigated. Longitudinal studies following large numbers of individual autism and control subjects over time are needed to determine how both amygdala and hippocampal volumes change relative to TBV with age within affected individuals.

7.4.2.3 Thalamus

Decreased thalamic volume has been found in autism participants ranging in age from late childhood into adulthood in studies controlling for total brain or intracranial volumes (Waiter et al. 2004; McAlonan et al. 2008; Tamura et al. 2010). Conversely, a number of studies show no mean volumetric differences in autism, but these studies did not control for the effects of global brain volumes (Tsatsanis et al. 2003; Hardan et al. 2006, 2008a, b; Haznedar et al. 2006). Previous volumetric studies provide conflicting results due to an atypical relationship between thalamic volume and TBV in autism: the positive relationship between thalamic volume and TBV in typically developing participants is absent in autism (Tsatsanis et al. 2003; Hardan et al. 2006, 2008a). Interestingly, a study by Tsatsanis et al. (2003) found that thalamic volumetric differences were greatest in the comparison between the autism and typically developing participants with the largest TBVs. Given the importance of the thalamus in sensory processing, further research into the development of this structure is warranted.

7.4.3 Cerebellum

Mean cerebellar volume is increased in very young children with autism but only when uncorrected for TBV. Thus, enlargement of the cerebellum seems to be part of the global increase in brain size at this stage of development. Between young childhood and mid-adulthood, structural abnormalities of the cerebellum are not universally present but likely affect a subgroup. The most consistent cerebellar changes in ASD from typical development are reduced gray matter (Abell et al. 1999; Salmond et al. 2003; Waiter et al. 2004; Cleavinger et al. 2008; Toal et al. 2010; Cauda et al. 2011; Yu et al. 2011; Ecker et al. 2012) and variable changes in the vermis, most commonly vermal hypoplasia and less frequently vermal hyperplasia. Volumetric alterations of white matter are less consistent (Boddaert et al. 2004). Note that diffusion tensor imaging, which can detect evidence of alteration of white matter microstructure in the absence of volumetric changes, finds some abnormalities (see DTI chapter). Readers very interested in the cerebellum in autism are referred to the excellent recent consensus paper, “Pathologic Role of the Cerebellum in Autism” (Fatemi et al. 2012).

Of the many structural MRI studies of the cerebellum, dating back to a seminal paper by Courchesne et al. (1988), we discuss a few recent noteworthy reports.

A meta-analysis found significant effect sizes for case–control studies of the left and right cerebellum (ES = 0.62 and 0.72, respectively) and vermal lobules VI to VII and VIII to X (ES = −0.27 and −0.43, respectively) (Stanfield et al. 2008). Age and IQ appeared related to heterogeneity in the effect sizes for vermal lobules VI to VII; older age and higher IQ were associated with attenuated case–control differences. Scott et al. (2009) performed a recent comprehensive study of cerebellar morphology in 62 male autistic subjects. All analyses included age and total cerebral volume as covariates. Analyses of cerebellar subregions tested these volumes unscaled and scaled (the ratio of subregion to total cerebellar volume). No ASD-control differences were found in left or right cerebellar hemispheres, including total volume, gray and white matter volumes, or in the medullary core. The only case–control difference was in the vermis; it was smaller in the ASD subgroup with high-functioning autism (i.e., without intellectual subnormality), but not in the subgroups with low-functioning autism or Asperger syndrome (Scott et al. 2009).

7.4.4 Region-Specific White Matter Abnormalities

7.4.4.1 Volumetric Studies of White Matter

Radua et al. (2011) recently performed a voxel-based meta-analysis testing for case–control differences in total and regional brain white matter. The method they used sensitively detects consistent case–control differences in an unbiased manner. No differences were found between older ASD and typically developing samples in total brain white matter. Regional differences were present in the ASD group. White matter was significantly increased in the ASD group in the right arcuate fasciculus, left uncinate fasciculus, and left inferior fronto-occipital fasciculus. When they divided the ASD sample into pediatric and adult subgroups, the same regional white matter case–control differences were found (Radua et al. 2011).

7.4.4.2 Corpus Callosum

The major white matter structure that has been studied in autism with conventional structural MRI is the corpus callosum, the major WM fiber system connecting the cerebral hemispheres. The corpus callosum is discrete, readily identifiable, and easily segmented using automated (or manual) methods. One of the most replicated findings in the autism literature is atypical morphometry of the corpus callosum (CC). Studies of CC structure report reduced mean area, volume, and white matter density in autism compared to typically developing individuals (Chung et al. 2004; Waiter et al. 2005; Alexander et al. 2007; He et al. 2008; Kilian et al. 2008; Casanova et al. 2009, 2011; Frazier and Hardan 2009; Freitag et al. 2009; Hardan et al. 2009b; Keary et al. 2009; Hong et al. 2011), with a few exceptions (Elia et al. 2000; McAlonan et al. 2002; Rice et al. 2005; Kilian et al. 2008; Tepest et al. 2010).

A meta-analysis of CC size in autism found the largest effect sizes for case–control differences in the anterior CC (Frazier and Hardan 2009). Work by Casanova and colleagues (2009) showed a reduced "gyral window," or space for afferent and efferent fibers to enter and exit the cortex, in autism. The reduced "gyral window" suggests a reduction in long-range cortico-cortical connections in the brain of individuals with autism (Casanova et al. 2009), and is consistent with decreased CC area and underconnectivity.

Further support for involvement of the CC in autism is based on impaired performance on neuropsychological tasks requiring more complex processing and interhemispheric information transfer (Minshew et al. 1997; Nyden et al. 2004). Studies using structural MRI have suggested a relationship between CC size and clinical features of autism (Hardan et al. 2009b; Keary et al. 2009). In addition, multimodal investigations have shown correlations between the size of callosal subregions and functional connectivity measured during tasks that tap cognitive skills frequently impaired or relatively preserved in the disorder (Just et al. 2004, 2007; Kana et al. 2006, 2009; Mason et al. 2008; Keary et al. 2009; Damarla et al. 2010; Schipul et al. 2011).

Research into the longitudinal development of the CC in autism is ongoing. Persistent volumetric reductions in autism during later childhood and adolescence in all CC subregions except the anterior body were found in a 2-year longitudinal MRI study (Frazier et al. 2012). Developmental studies of typical individuals show that CC development begins in utero and continues into young adulthood (LaMantia and Rakic 1990; Pujol et al. 1993; Keshavan et al. 2002; Giedd 2004; Giedd et al. 2006). Myelination of the CC begins in posterior regions at 3–4 postnatal months and in anterior regions at 6–8 postnatal months (Deoni et al. 2011). Subsequently, in typical development, growth of midsagittal CC area proceeds from an anterior to posterior direction, which is in contrast to the posterior to anterior directionality of cortical maturation (Jancke et al. 1999; Giedd 2004). Longitudinal MRI studies confirm this anterior–posterior development and show that the greatest callosal changes during childhood and adolescence occur in the posterior regions (Giedd et al. 1999; Thompson et al. 2000). Future longitudinal studies of the neurodevelopment of the CC in autism will show whether abnormal CC structure is present from birth or emerges during development.

7.5 Gender Differences

The overwhelming majority of neuroimaging research has focused on males with autism because of the sex differences in autism prevalence. In the few studies that have focused on females with autism and compared their structural brain anatomy to typically developing females, differences have been found. Craig et al. (2007) did a VBM analysis to compare women with autism and a matched female control group. They found that women with autism have less gray matter extending throughout the temporal lobe, the orbitofrontal cortices, anterior cingulate cortex, and

cuneus. The women with autism also have less white matter in the anterior temporal lobe and brain stem, whereas they have more white matter in the splenium of the CC, the anterior cerebellum, and also throughout the temporal, frontal, and parietal lobes. In another VBM study of young girls, it was found that girls with autism have significantly more gray matter in both superior frontal gyri and the right temporoparietal junction (Calderoni et al. 2012).

In addition to identifying neurobiological differences between females with autism and typically developing females, it is important to understand how the neurobiological correlates of autism differ between males and females. Bloss and Courchesne (2007) found similar differences when comparing young girls and boys with autism to their respective sex-matched control groups. The two groups with autism had similar differences (i.e., enlargements of whole brain, cerebral gray matter, cerebellar white matter, and frontal gray matter); however, the girls with autism had additional abnormalities, namely, more temporal lobe gray matter and intracranial volume and less cerebellar gray matter than the typically developing girls (Bloss and Courchesne 2007). The boys with autism had more white matter in the frontal lobe (Bloss and Courchesne 2007). In another study, women with autism had less gray matter in the cerebellum, the posterior cingulate, the amygdala, and the parahippocampal gyrus than a group of males with autism matched on disorder severity (Murphy et al. 2011). Finally, Beacher et al. (2012) found that male controls have larger total white matter volumes and in the right inferior parietal lobe than female controls. This sexual dimorphism was attenuated in autism, in the case of the total white matter volume, and absent in autism, in the case of the right inferior parietal lobe (Beacher et al. 2012). These initial studies must be followed up by additional studies to confirm sex differences in autism and differences in brain structure between females with autism and typically developing females.

7.6 Predicting Phenotype from Brain Structure

7.6.1 Structural Imaging as a Probe for Phenotypic Subtyping

Characterizing autistic subtypes using structural neuroimaging techniques remains a major goal in the field. Jiao et al. (2010) recognized this wide phenotypic range and employed both volumetric and thickness-based analyses to construct predictive models in a small number of subjects aged 6–15 years. Using predefined atlas-based anatomic parcellations, they employed four machine-learning algorithms to generate diagnostic models, and found that learning model tree approaches could classify autistic subjects using only seven regions with a sensitivity of 95 % and specificity of 75 %. Volume-based assessment was inferior, implying that surface area (versus thickness) may be less helpful in predicting diagnosis. They describe decreased cortical thickness in ASD within bilateral IFG (pars triangularis), and left medial orbitofrontal gyrus, parahippocampal gyrus, and frontal pole, and increased thickness in left caudal anterior cingulate and precuneus.

Misaki et al. (2012) utilized a unique approach to this problem by framing their analysis of cortical thickness to "canonical patterns" between ASD and controls. Their model showed that IQ is positively correlated with cortical thickness in orbitofrontal, postcentral, and superior temporal regions in adolescents. Further, greater thinning with age was seen in dorsal frontal regions in those with "superior IQ" (>120). This is not inconsistent with earlier work in normal controls (Shaw et al. 2006). However, neither of these associations were seen in the ASD group. This suggests that the structural correlates of the same IQ score are altogether different in autism versus normal adolescents.

Several recent studies using novel types of analysis have tried to determine if the structural neuroanatomy of autism and Asperger syndrome differ. Toal et al. (2010) VBM study investigated 65 adults with ASD (39 Asperger syndrome, 26 Autism) compared to 33 typically developing adults. The subgroup with autism, but not the Asperger syndrome subgroup, had reductions in frontal and temporal gray matter compared to the typical controls. The results suggested "that anatomical difference from typical development differ according to the clinical subtypes" (Toal et al. 2010). The results have not, however, been replicated. Via et al. (2011) performed a VBM meta-analysis of 20 published studies. They did not find any global or regional GM volumetric differences between autism and Asperger. Yu et al. (2011) also completed a meta-analysis of 15 studies. Although they did not directly compare autism to Asperger, they found distinct patterns of GM volumetric differences in each group compared to typically developing samples.

7.6.2 What Do Structural MRI Results Suggest About Mechanisms Involved in ASD?

The autism literature is converging on general principles of developmental changes in structural brain anatomy. Head circumference and gross brain morphology undergoes early excessive expansion, led by increasing head circumference in early postnatal life followed by increasing brain size sometime after 6 months but before 2.5 years. Anterior brain regions may be affected first. Brain growth then enters a phase of deceleration and plateau, which may occur earlier in girls than boys. Brain growth "pseudonormalizes" by mid-childhood, and decreases in compartmental brain volumes are seen in late adolescents and young adults. This period of decline may extend, or even accelerate, into mid-adulthood and beyond. The differential impact of white matter changes versus gray matter changes could teach us much about underlying cellular and molecular mechanisms underlying ASD. Similarly, cortical assessments such as thickness, surface area, and volume may reflect different time points or locations of abnormal neurodevelopmental origins. Heterochronicity and spatial heterogeneity among autistic subjects is likely related to differential phenotypic expression of disease characteristics. Similarly, more fine-grained analyses of regional specificity or selectivity across ages, demographics, autistic subtypes, and assessment techniques will provide further targets to guide molecular, cellular, and genetic studies.

Macroscopic structural changes by themselves will not fully explain the pathogenic mechanisms of autism; the ultimate determinant of macroscopic structure is underlying molecular and cellular processes driven by genetic abnormalities, refined by functional trophism, and sculpted by cognitive, social, and environmental feedback. The process is reciprocal and iterative, and although gross abnormalities may comprise a common thread of the class of autistic pathologies, detailing the full complement of the phenotypic expression in autism necessitates a comprehensive understanding of how each of these factors influences, and is influenced by the others. Outstanding questions remain to be answered. Do regional growth trajectories predict phenotype? Can we relate regional changes to functional domains? Is there a pattern among patterns of structural change? As technology improves, and techniques become increasingly more sophisticated, the field will begin to move beyond the study of gross anatomical structural relationships, instead focusing on phenotypic relationships to these structural abnormalities.

7.7 Conclusion

Autism is a clinical syndrome; a feature set of phenotypically variable deficits in socio-communicative, movement, and hedonic processing. It remains a diagnosis based on behavioral assessment, with no robust or reliable objective "biological markers." Structure begets function on all levels of neurobiology, from ligand-gated ion channels to large-scale brain networks, and the structure of the autistic brain holds important clues to the origins and manifestations of the disease. Although the degree of inconsistency in the literature to date can be discouraging to some, identifying commonalities upon which to build broader interpretive context will frame a new understanding of autistic neurobiology.

A story is emerging of variable and perhaps independent trajectories of abnormal brain structural dynamics over time, influenced by demographic, severity, age, developmental stage, and cognitive capabilities. Cross-sectional studies can transect one plane of this multidimensional system, and while they can provide critical observations, must be interpreted in context. Similarly, longitudinal studies can clarify within-subject changes over time, but here again careful attention must be placed to region, age, stage, and phenotypic subtype of subjects in order to be correctly interpreted. It needs no mention that further research remains to be done. As the explosive body of work in the macroscopic structure of autism accelerates further, the focus will shift from gross morphological trends to specific regional assessments of phenotype–genotype relationships, enabling new research and treatment strategies focused by this new neurobiological framework. Indeed, future scientists, clinicians, and families may not refer to "autism" as a diagnostic entity, but rather "the autisms" as a category of distinct but related entities comprising differential phenotypic, neuroimaging, and genetic features, and ultimately, therapeutic successes (Table 7.5).

Table 7.5 Glossary of MRI terminology

General neuroimaging terminology	Description/definition
CSF	Cerebrospinal fluid (CSF) is the fluid that bathes the central nervous system. It surrounds the exterior of the brain and spinal cord and occupies the venous sinuses and ventricles, or spaces on the interior of the brain and brain stem. CSF maintains a constant environment for central nervous system tissue and is essential for removing harmful metabolites. CSF also acts as a cushion between the brain and skull
GM	Gray matter (GM) is any portion of tissue in the nervous system that is predominantly occupied by the soma, or cell body, of neurons (e.g., the cortex of the cerebrum)
WM	White matter (WM) is any portion of tissue in the nervous system that is predominantly occupied by the axons of neurons [e.g., any white matter tract or fasciculus (the corticospinal tract that connects the motor cortex with the neurons in the spinal cord that signal to the skeletal muscles to move)]
T1, T2	A main goal of MRI is to generate contrast between different types of tissues. This is performed using radiofrequency pulses and spin–lattice relaxation. Before any radiofrequency waves are applied, the overall magnetic field aligns the spinning protons along the axis that spans the length of the scanner (i.e., the longitudinal axis), which in most cases corresponds with the length of participant. Then when a radiofrequency pulse is administered, some of the spinning protons flip 90^{o} from the longitudinal axis to the transverse axis, which is perpendicular with the length of the scanner and the participant. Over time, the protons that have been flipped to the transverse axis return to the longitudinal axis, or "relax," and emit radiofrequency energy that is detected by the MR scanner T1 is a time constant that corresponds to the amount of time it takes for the longitudinal magnetization to return. Brain tissue has shorter T1 relaxation than CSF T2 is a time constant that refers to the amount of time it takes for the transverse magnetization to decay, and is always less than or equal to T1. WM has a shorter T2 than GM
TE, TR	Time of echo or echo time (TE) is the amount of time from the radiofrequency wave pulse administration to the signal acquisition or the time when the image is captured. Time of repetition or repetition time (TR) is the amount of time from one pulse of radiofrequency waves to the next pulse
Field of view, slice thickness, flip angle	The field of view is the extent of the participant that is observable by an MR image. The slice thickness is the dimension of the *z* axis for a single section or image of the entire imaging volume. The flip angle is the change in angle of magnetization from the main magnetic field caused by the radiofrequency pulse administered
T2-weighted	An image that is more dependent on the T2 properties than T1 properties
Proton density	An imaging sequence that has a long TR and a short TE. Therefore, it has the higher signal-to-noise ratio than T1- or T2-weighted images. GM and CSF show up with a similar contrast in proton density images, whereas WM is brighter than GM and CSF

(continued)

Table 7.5 (continued)

General neuroimaging terminology	Description/definition
SPGR	Depending on the imaging sequence, residual transverse magnetization may exist from a previous radiofrequency pulse, which would interfere with the subsequent radiofrequency pulse and image acquisition. Spoiled gradient recalled is an imaging sequence that "spoils" or dephases the residual transverse magnetization
Echo time	TE (see above)
Pulse sequence	The pulse sequence is a series of radiofrequency pulses applied to the participant that produce a specific signal
MATLAB®	MATLAB is short for matrix laboratory and is a software package and computer language developed by The MathWorks Inc. (http://www.mathworks.com). It is amenable to neuroimaging toolboxes, such as statistical parametric mapping (SPM), to process and analyze imaging data
Tissue segmentation	Partitioning of an image according to tissue type. It typically includes separating GM, WM, CSF, and surrounding soft tissues from one another
Region of interest (ROI)	The area or volume of the nervous system that is studied or examined in a neuroimaging experiment
Partial volume	When signal is measured from a region that is much smaller than the dimensions of an image's voxel or pixel, an apparent loss in signal occurs. This loss in signal is defined as the partial volume effect
Voxel	Voxel is short for a volume element. It is a three-dimensional volume of any given size (e.g., 3 mm × 3 mm × 3 mm), and together, many voxels make up an entire three-dimensional image
Pixel	Pixel is short for a picture element. It is a two-dimensional area of any given size (e.g., 1 mm × 1 mm). Together, many pixels make up an entire two-dimensional image

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Chapter 8
Diffusion Tensor Magnetic Resonance Imaging in Autism

Brittany G. Travers and Andrew L. Alexander

8.1 Introduction

In this chapter, we describe the methods of diffusion tensor imaging (DTI), and we describe how these imaging methods have been applied to the study of autism spectrum disorder (ASD). This chapter extends upon a recent review of the literature (Travers et al. 2012), by providing information on more recent studies and in-depth information regarding the WM integrity of the cerebellar, frontal, and thalamic tracts. Therefore, we first review DTI studies of young children with ASD because these children are closest in age to the primary, initiating pathology of autism. Then, we present and discuss results from particular WM tracts and brain regions of high interest from childhood into adulthood. Specific factors that may potentially influence and at times confound case–control differences in DTI studies are mentioned. We then discuss what DTI findings to date suggest about biological mechanisms involved in autism. Results from the reviewed studies are included in Table 8.7, which is an extension of the table presented in the review by Travers et al. (2012).

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8.2 Diffusion Tensor Imaging

DTI (Basser et al. 1994b) is a noninvasive magnetic resonance imaging (MRI) method that can be used to map and characterize the organization of brain tissues by tracking the diffusion of water (Jones et al. 1999b; Mori et al. 2002; Catani and Thiebaut de Schotten 2008). Water diffusion is the random dispersion of water molecules, and it is affected by the microstructural and macroscopic organization of the surrounding tissue. In other words, the diffusion of water is highly sensitive to and modulated by the brain matter that surrounds it. Therefore, if we can follow how the water diffuses across brain tissue over time using DTI, we can gain information regarding tissue-specific microstructure and macrostructure.

Because water diffuses differently in white matter (WM) compared to gray matter, DTI methodology can be used to distinguish WM tracts from the surrounding gray matter. WM tracts consist of bundles of axons that serve as the "highways of the brain" and allow for efficient communication between brain regions. The fibrous tissue structure of axon bundles restricts the diffusion of water in the direction perpendicular to a WM tract more so than in the direction parallel to the WM tract. This restriction increases the diffusion that goes along the tract, causing a directional dependence of water diffusion called anisotropic diffusion. In other words, anisotropic diffusion is when water diffuses primarily in one direction (presumably along a WM tract), compared to isotropic diffusion, which is when water diffuses equally in all directions (often occurring in cerebrospinal fluid and gray matter). Changes to the microstructural properties of WM, such as myelination, axonal density, and axonal caliber, influence diffusion anisotropy and may reflect differences in brain structural connectivity.

DTI models anisotropic water diffusion by the signal attenuation in images with diffusion-weighted gradients applied in the directions of interest. A minimum of six noncollinear encoding directions of diffusion weighting are necessary to estimate the full diffusion tensor (Shrager and Basser 1998; Papadakis et al. 1999). However, a wide variety of diffusion-tensor encoding strategies with often more than six encoding directions have been used (Basser and Pierpaoli 1998; Jones et al. 1999a; Papadakis et al. 1999; Shimony et al. 1999). Encoding schemes with the most uniform angular distributions tend to provide the most accurate results (Hasan et al. 2001). There is evidence that more encoding directions diminish the variability of DTI measures as a function of tensor orientation (Batchelor et al. 2003; Jones 2004).

8.2.1 Diffusion Tensor Imaging: The Mathematical Model

Basser et al. (1994b) developed an elegant tensor model to describe the anisotropic diffusion behavior of the water signal in biological tissues. (A tensor is a generalized mathematical form of a vector involving a number of indices.) The diffusion tensor models water diffusion as a three-dimensional (3D) Gaussian distribution, a 3×3 matrix (with elements D_{ij}), which describes the relative diffusion in a 3D coordinate system (Basser et al. 1994a, b; Basser 1995; Basser and Pierpaoli 1996). Since this matrix is diagonally symmetric, six independent variables comprise the diffusion tensor.

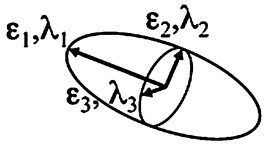

Fig. 8.1 Illustration of the diffusion tensor ellipsoid representing anisotropic diffusion in an area of WM

It is important to represent the six-dimensional data in a meaningful way for interpretation. A convenient graphical way to represent the diffusion tensor is by a 3D ellipsoid as shown in Fig. 8.1. This 3D ellipsoid can indicate the direction and strength of the water diffusion at each pixel location of the brain image. The spatial orientation or direction of the ellipsoid corresponds to the eigenvectors ($\boldsymbol{\varepsilon}_1$, $\boldsymbol{\varepsilon}_2$, $\boldsymbol{\varepsilon}_3$) of the diffusion tensor. The length of each eigenvector is described by the eigenvalues (λ_1, λ_2, λ_3) of the diffusion tensor. Along a WM tract, the largest axis of the ellipsoid is defined by the major eigenvector ($\boldsymbol{\varepsilon}_1$) and eigenvalue ($\lambda_1$) and is parallel to the direction of the WM fibers. The directions perpendicular to the WM fiber tracts are defined by the intermediate and smallest axes of the diffusion tensor ellipsoid described by the medium and minor eigenvectors ($\boldsymbol{\varepsilon}_2$, $\boldsymbol{\varepsilon}_3$) and eigenvalues ($\lambda_2$, λ_3).

8.2.2 Diffusion Tensor Imaging: Measures of the Physical Properties of Tissue Microstructure and Macrostructure

Fractional anisotropy (FA) is a normalized standard deviation of the diffusion tensor eigenvalues that characterizes the directional variation in the apparent diffusion (Basser and Pierpaoli 1996),

$$\mathrm{FA} = \sqrt{\frac{3}{2}\frac{(\lambda_1 - \mathrm{MD})^2 + (\lambda_2 - \mathrm{MD})^2 + (\lambda_3 - \mathrm{MD})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}, \tag{8.1}$$

where MD is the mean diffusivity defined by the average of the three eigenvalues.

$$\mathrm{MD} = \frac{(\lambda_1 + \lambda_2 + \lambda_3)}{3} \tag{8.2}$$

FA is the most ubiquitous DTI measure and is a normalized standard deviation of the eigenvalues; FA ranges from 0 to 1 with smaller FA in more isotropic tissues (i.e., gray matter and cerebrospinal fluid) and higher FA in regions of WM. Higher FA values are indicative of more elongated and skinnier ellipsoids, with the greatest diffusion parallel to the tract. In contrast, lower FA values are indicative of more spherical ellipsoids, suggesting more even diffusion among the three directions. FA is highly sensitive to microstructural changes or differences in WM including myelination and axonal density and therefore is often called a measure of WM integrity or structural connectivity.

Although FA is the most popular DTI measure, FA cannot provide a complete description of WM microstructure (Alexander et al. 2000), and therefore, additional DTI measures should be investigated in order to better interpret the underlying changes in microstructure of the tissue (Alexander et al. 2007). Additional DTI measures include mean diffusivity (MD), axial diffusivity, and radial diffusivity. MD (Eq. 8.2), the average radius of the diffusion tensor ellipsoid, is sensitive to the density of tissue barriers in all directions. Axial diffusivity (AD) is the length of the first (longest) eigenvalue of the tensor ($AD = \lambda_1$). Radial diffusivity (RD), also known as the perpendicular diffusivity, is the average of the second and third eigenvalues ($RD = (\lambda_2 + \lambda_3)/2$) and measures water diffusion perpendicular to the WM tract. RD is thought to be indicative of myelin integrity in animal models of dys- and demyelination (Song et al. 2002, 2005; Harsan et al. 2006; Tyszka et al. 2006). However, changes in density or diameter of the axons, changes in cytoskeletal properties, or swelling from neuroinflammation are also plausible explanations for changes to RD and other DTI measures (including FA). Therefore, caution must be used to not overinterpret DTI changes in these measures (Wheeler-Kingshott and Cercignani 2009).

A significant limitation of DTI is that it is inadequate for describing WM microstructure in regions where there are crossing WM fibers (Alexander et al. 2001a; Wedeen et al. 2008). This often occurs in regions of crossing fibers which make the diffusion tensor more isotropic. Thus, FA is reduced in regions with complex WM fiber crossings. Currently, new methods (diffusion spectrum imaging, high angular resolution diffusion imaging) are being developed to try to characterize crossing tracts in DTI (Wedeen et al. 2008; Tournier et al. 2011). However, until these new methods are used to study autism, some caution should be used in the interpretation of DTI measures in the WM tracts that are known to have a number of crossing fibers.

8.2.3 DTI Analysis: Region of Interest, Voxel-Based, and Tractography-Based Approaches

WM in the brain is heterogeneous. WM fibers coalesce to form discrete tracts in some compartments of the brain, whereas in other areas, such as within gyri, discrete tracts may not be discernible. The heterogeneity of WM across the brain necessitates the application of brain region-specific DTI analysis methods. Techniques for investigating individual and group differences in FA, MD, AD, and RD include region of interest (ROI), voxel-based analysis (VBA), and tractography-based approaches.

8.2.3.1 VBA Approach

Methods for statistical parametric mapping (SPM) have become popular for analyzing DT-MRI data for both within and between case–control groups. The approach is similar to methods for voxel-based morphometry (VBM) (Ashburner and Friston 2000). Brain image DTI maps are spatially co-registered (normalized) to a brain

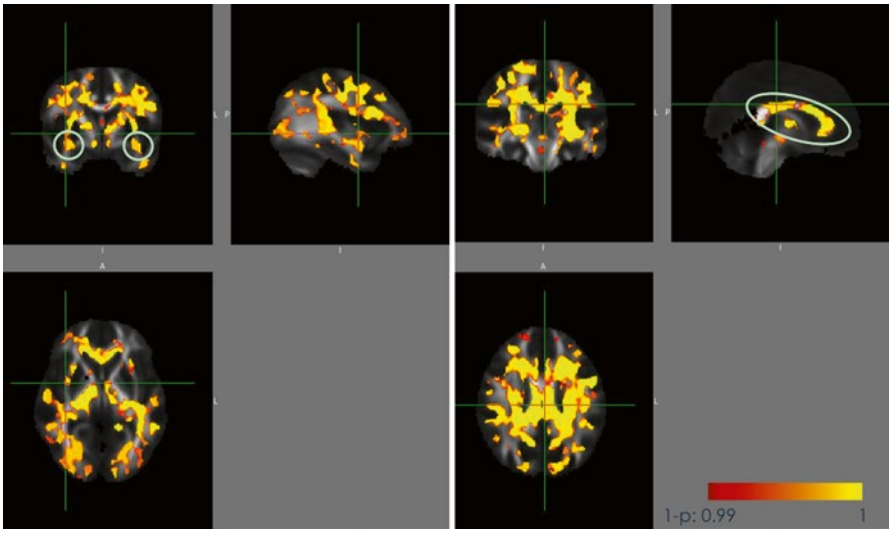

Fig. 8.2 DTI voxel-based approach (VBA) depicting spatially diffuse increases in λ_3 (the smallest perpendicular eigenvalue) in ASD (Alexander AL et al., Oral presentation, IMFAR, 2009). Samples sizes were 43 ASD and 34 TD controls. The results were generated using T-SPOON (Lee et al. 2009) with nonlinear spatial normalization, 6 mm of smoothing, and an age-corrected ANOVA with significant differences shown for $p<0.01$ (corrected)

template and a local smoothing kernel is applied. Then, statistical testing is performed at each voxel location within the brain. A voxel-by-voxel statistical test (ANOVA, General Linear Model, etc.) is used to determine what brain regions exhibit consistent differences in the FA maps across subjects (Lee et al. 2009). As shown in Fig. 8.2, the resultant VBA statistical maps often exhibit spatial "blobs" of significance on a normalized brain map.

The VBA approach is attractive because the analysis is automated and does not require hours of tedious ROI drawing. It is performed over the entire brain, such that significant differences in areas not anticipated a priori can be detected. Moreover, software tools exist to do the analysis (e.g., SPM, by Friston and colleagues at the University College of London; http://www.fil.ion.ucl.ac.uk/spm/), and the statistical methods for the approach have already been developed for analysis of fMRI and morphometry data. Voxel-based SPM analysis of DTI data has been applied to a broad spectrum of neuropsychiatric disorders (Buchsbaum et al. 1998; Foong et al. 2000; Agartz et al. 2001; Ardekani et al. 2003; Barnea-Goraly et al. 2003a, b, 2004), to children with poor reading ability (Klingberg et al. 1999), and to brain development in typically developing children (Schmithorst et al. 2002).

There are several limitations of voxel-based SPM methods. A potential confound in the SPM approach is imperfect spatial registration between individuals; thus, differences in brain morphology could manifest as apparent regional differences in FA between subjects. Improvements to image registration algorithms may improve the confidence in SPM studies. Spatial normalization may also increase image blurring.

One unique aspect of VBA specific to DTI is that the orientation of the diffusion tensor may be rotated to the same orientation as the target image or template space (Alexander et al. 2001b). Several approaches to the registration of DTI images with tensor transformation have been reported (Alexander et al. 2001b; Jones et al. 2002; Park et al. 2003; Xu et al. 2003). Another limitation of the SPM approach is that the case–control analyses have considered only a single scalar parameter, FA, whereas the diffusion tensor is a multidimensional entity. The use of multivariate statistical models could potentially improve the detection of group differences and increase the flexibility in developing models to describe the interactions between variables. Finally, the large numbers of voxels in DTI images lead to multiple comparisons (e.g., testing statistical significance at every voxel), which increase the likelihood of false positives. Few, if any, DTI SPM studies have corrected their results for multiple comparisons. Recently, VBA has been improved through better spatial normalization methods, e.g., DTI-TK (Zhang et al. 2007) and methods for decreasing the effects of image blurring and multiple comparisons, e.g., T-SPOON (Lee et al. 2009) and TBSS (Smith et al. 2006).

8.2.3.2 ROI Approach

Brain regions of interest may be regionally segmented either by manually outlining the region or by aligning a template to the image. Manual tracing of the ROI may be prone to user variability. Automated regional segmentation template methods take less time than manual tracing. However, automated segmentation can result in errors from misalignment due to inadequate spatial normalization. One strength of ROI methods is that voxels of interest are combined in a manner that is a priori defined, thus reducing the number of statistical tests performed and decreasing the chances of false positives. Figure 8.3 shows an example of a ROI segmentation approach in a DTI study of the superior temporal gyrus and temporal stem in autism (Lange et al. 2010).

8.2.3.3 Tractography-Based Approach

White matter tractography (WMT) methods offer a unique opportunity to segment WM pathways in the brain in vivo. The structural parcellation of the WM is based upon apparent connectivity patterns. WMT methods generate anatomically plausible tract reconstructions of major WM pathways in the human brain (Fig. 8.4) (Basser et al. 2000; Poupon et al. 2000; Stieltjes et al. 2001; Catani et al. 2002; Mori and van Zijl 2002; Xu et al. 2002).

Tractography methods reconstruct WM pathways, which may be used to define ROIs (Conturo et al. 1999; Mori et al. 1999). Tractography estimates WM connectivity using the directional information of the diffusion tensor. To reconstruct a WM tract, a streamline algorithm follows principal directions of the tensors. Tractography has been used to define major WM ROIs (e.g., cingulum, corpus callosum) (Conturo et al. 1999; Mori et al. 1999; Hofer et al. 2008). The methods can also estimate tract length and density. Most algorithms use the largest eigenvector to estimate the trajectory (Conturo et al. 1999; Mori et al. 1999; Basser et al. 2000).

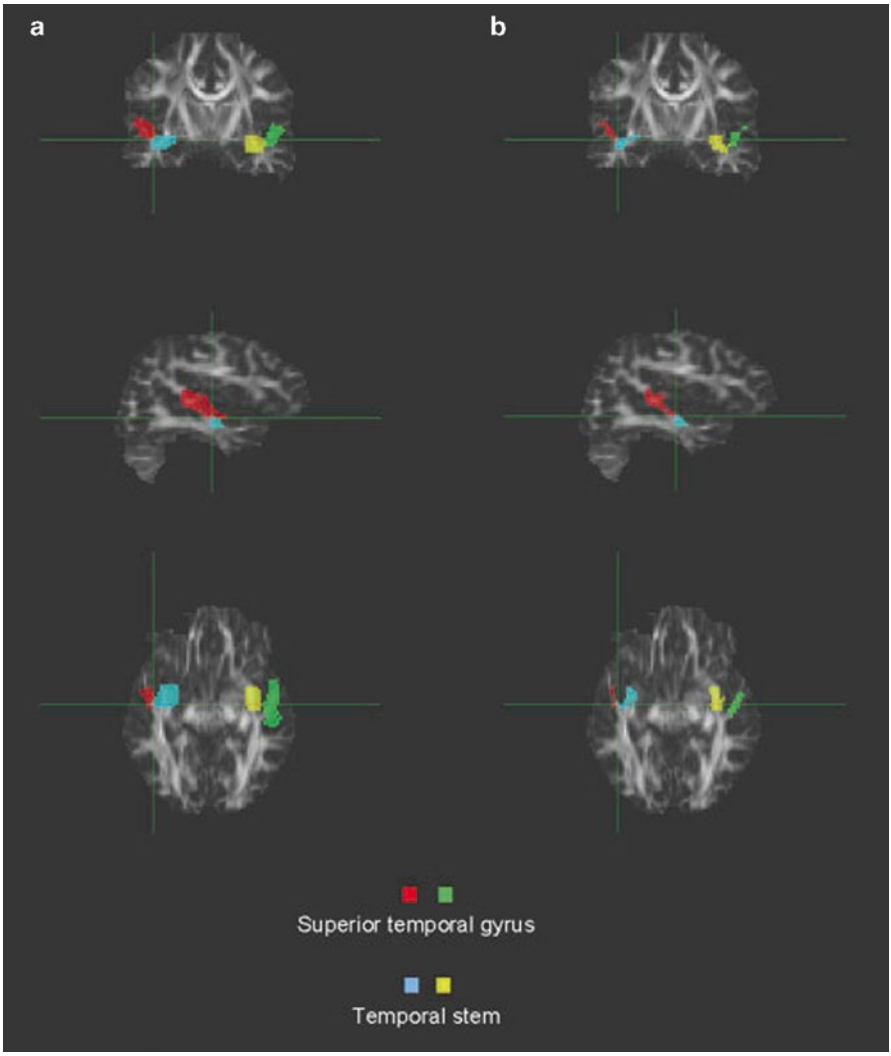

Fig. 8.3 DTI region of interest (ROI) approach. Example of segmentation of the superior temporal gyrus and temporal stem. (**a**) Raw. (**b**) Masked for WM only (Lange et al. 2010)

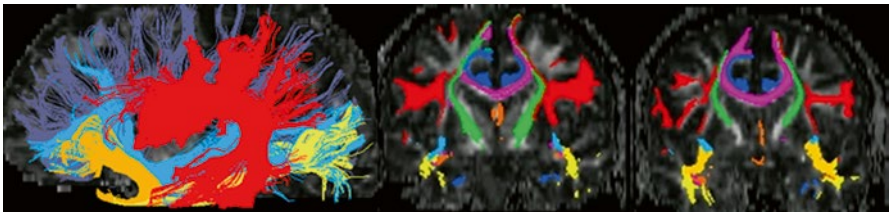

Fig. 8.4 WM tractography parcellation of major WM tracts for one subject: superior longitudinal fasciculus (*red*), corpus callosum (*purple*), inferior occipital fasciculus (*light blue*), inferior longitudinal fasciculus (*yellow*), uncinate fasciculus (*orange*), fornix/stria terminalis (*dark orange*), projection fibers of corona radiata (*green*)

WMT accuracy is influenced by a number of factors. Measurement noise also causes errors in the DTI eigenvector directions (Anderson 2001; Lori et al. 2002; Tournier et al. 2002; Lazar and Alexander 2003), though these errors are relatively small in homogeneous WM pathways. However, both DTI and tractography are inadequate for describing the fiber architecture in regions of complex WM with crossing fibers.

8.3 DTI Measurements in Developmental and Neuropsychiatric Disorders

It is possible that many developmental disorders, including autism, have abnormalities in brain WM that contribute to cognitive deficits, behavioral aberrations, and complex emotional and social disturbances. Generally, these WM abnormalities are difficult to detect and characterize using conventional imaging methods. Many investigators use DTI as a tool to probe WM differences in groups of patients with disorders such as developmental delay (Filippi et al. 2003), leukodystrophies (Guo et al. 2001; Schneider et al. 2003), fragile X (Barnea-Goraly et al. 2003b), velocardiofacial syndrome (Barnea-Goraly et al. 2003a), schizophrenia (Buchsbaum et al. 1998; Lim et al. 1999; Foong et al. 2002; Kubicki et al. 2003), and other developmental and neuropsychiatric disorders.

By modeling the diffusion of water as an ellipsoid at each voxel of the brain, we can calculate fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). Increased FA is thought to be indicative of increased fiber coherence, whereas increased MD is thought to be indicative of decreased tissue density. Axial diffusivity measures the diffusion parallel to the WM tract, whereas RD measures the diffusion perpendicular to the WM tract.

DTI is a neuroimaging technique that provides in vivo measurements of WM integrity by imaging the diffusion of water across the different tissues of the brain.

DTI measures can be used to segment WM tracts of the brain by propagating streamlines from voxel-to-voxel (tractography). Additionally, group differences in DTI measures can be analyzed using a priori defined WM regions of interest (ROI approach) or by using whole-brain voxel-by-voxel comparisons (VBA approach).

In the majority of studies with children, adolescents, and adults, findings have typically demonstrated decreased FA in ASD. However, in the three studies of very young children with ASD, findings have demonstrated an opposite pattern (increased FA in ASD).

8.4 DTI Studies of Autism Spectrum Disorders

Since the first study in 2004, there has been an ever-increasing use of DTI methodology applied to the study of ASD (Fig. 8.5). Despite the frequency of these studies, the sample sizes of the ASD and comparison groups have tended to be small

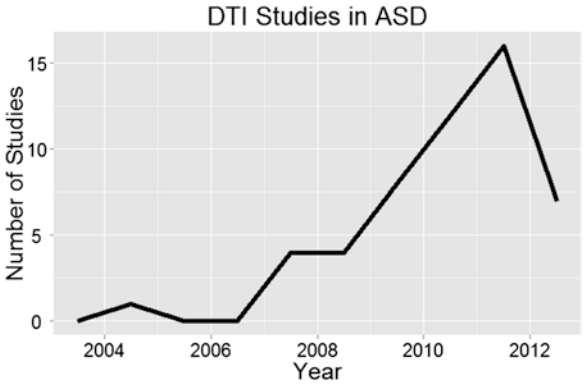

Fig. 8.5 Frequency of diffusion tensor imaging in autism studies since the first study in 2004

(Travers et al. 2012). Indeed, even with the four additional DTI studies that have been published since Travers et al. reviewed the literature, the average sample size of the ASD group has been 20.8, and 41 % of the studies had 15 or fewer participants with ASD. These small sample sizes suggest that we should interpret many of the results with caution.

8.4.1 DTI Research of Very Young Children with ASD

The results of only a few DTI studies of very young ASD children and infants who were later diagnosed with ASD are published. Additional studies are in progress. The published results to date are in striking contrast to the results of most DTI studies of older children and adults with ASD. As discussed below, in older ASD individuals, when an ASD-control difference in FA was found, it was almost always a decrease in FA (suggesting poorer integrity of WM microstructure in ASD). Differences in FA in very young children with ASD are mostly in the opposite direction; FA is increased.

Table 8.1 summarizes the results of the two largest studies of very young children with ASD. Young ASD-affected children were compared to high risk but unaffected siblings of children with ASD in one of the studies (Wolff et al. 2012). In the other studies, the comparison group was pediatric patients referred for clinical evaluation for a variety of concerns but found to have normal conventional MRIs (Ben Bashat et al. 2007; Weinstein et al. 2011). The only WM tract found to have an increase in mean FA in both studies was the corpus callosum. The specific subregion affected was the body of the corpus callosum. It should be noted, however, that not all tracts were examined in both studies.

Weinstein et al. (2011) reported increased mean FA in the genu and body of the corpus callosum, left and right cingulum bundles, and left superior longitudinal fasciculus in young ASD children. Wolff et al. (2012) found increased mean FA in the body of the corpus callosum, the left fornix, left inferior longitudinal fasciculus,

Table 8.1 Mean fractional anisotropy (FA) in infants and very young children with ASD

	Wolff et al. (2012) $N=28$ High-risk infants who developed ASD (compared to $N=64$ high-risk infants who did not develop ASD)				Weinstein et al. (2011) $N=21$ Children with autism (compared to $N=26$ pediatric patient infants with normal conventional MRIs)
Tesla, b-value (s/mm^2)	3.0 T, $b=1{,}000$				1.5 T, $b=1{,}000$
DTI analysis method	Tractography—mean FA of entire tracts				Tract-based spatial statistics—voxels with significant case–control differences within tracts
	Age 6 months	12 months	24 months	Rate of growth from 6 to 24 months	Mean age 3.3 years (1.5–5.8 years)
Ant. limb internal capsule					
Left	No difference	No difference	Decreased FA	Lower rate of increase in FA	No TBSS difference
Right	No difference	No difference	No difference	Lower rate of increase in FA	No TBSS difference
Ant. thalamic radiation					
Left	No difference	Decreased FA	Decreased FA	Lower rate of increase in FA	No difference
Right	No difference	No difference	No difference	No difference	No difference
Corpus callosum					
Genu	No difference	No difference	No difference	No difference	Increased FA
Body	Increased FA	No difference	No difference	Lower rate of increase in FA	Increased FA
Splenium	No difference	No difference	No difference	No difference	No difference
Fornix					
Left	Increased FA	No difference	No difference	Lower rate of increase in FA	No difference
Right	No difference	No difference	No difference	Lower rate of increase in FA	No difference

Inf. longitudinal fasciculus					
Left	Increased FA	No difference	No difference	Lower rate of increase in FA	No difference
Right	No difference	No difference	No difference	Lower rate of increase in FA	No difference
Post. limb internal capsule					
Left	No difference	No difference	No difference	Lower rate of increase in FA	No difference
Right	Increased FA	No difference	No difference	Lower rate of increase in FA	No difference
Uncinate					
Left	Increased FA	No difference	No difference	Lower rate of increase in FA	No difference
Right	No difference	No difference	No difference	No difference	No difference
Cingulum					
Left	NA	NA	NA	NA	Increased FA*
Right	NA	NA	NA	NA	Increased FA*
Superior longitudinal fasc.					
Left	NA	NA	NA	NA	Increased FA*
Right	NA	NA	NA	NA	No difference

NA not examined

*$p < 0.05$ only when uncorrected for multiple comparisons

right posterior limb of the internal capsule (with a trend on the left), and left uncinate fasciculus of affected infants. The increased mean FA found by Wolff et al. (2012) was temporary. FA was increased when the affected infants were 6 months of age but not when they were 12 and 24 months old. Several tracts, not affected when the ASD children were 6 months of age, had *decreased* FA when the children were older. Mean FA was decreased in the left anterior thalamic radiations when the affected children were 12 and 24 months of age and in the anterior limb of the left internal capsule when the children were 24 months of age.

Longitudinal analysis of the DTI data by Wolff et al. (2012) showed that after the increase in FA at 6 months of age, the rate of developmental increase in FA between 6 and 24 months of age was less than typical in the ASD children. Cross-sectional age-related changes in the Weinstein et al. (2011) study suggested a more sustained increase in FA in early childhood in autism. Cross-sectional data, however, do not measure development within individuals but infer the development by examining changes across individuals. Therefore, cross-sectional age-related results always need to be confirmed by longitudinal investigation.

It is important to note that not all affected infants had increased FA at 6 months of age in the study by Wolff and colleagues (2012), and there was a substantial overlap between FA values for affected and unaffected children. In addition, the proportion of affected children with abnormal trajectories of FA was not provided. Interindividual variability in FA at all ages appeared significant. The interindividual variability and the relatively small sample of very young affected children suggest that the results need to be considered with caution and they need to be validated by replication. These results of these studies are, nevertheless, very important.

8.4.2 Age-Related Changes in WM Microstructure: Are They the Same in ASD and Typical Development?

The fact that the DTI studies with the youngest samples have typically found the opposite pattern of results (i.e., increased FA in ASD) compared to the rest of the body of literature suggests the importance of understanding how WM microstructure develops with age in both individuals with ASD and individuals with typical development. In individuals with typical development, WM growth and myelination develops from a posterior-to-anterior gradient with age (Yakovlev and Lecours 1967). Correspondingly, individuals with typical development demonstrate increased volume, increased FA, and decreased MD in most WM tracts from childhood into adulthood (Barnea-Goraly et al. 2005; Ashtari et al. 2007; Giorgio et al. 2008, 2010; Lebel et al. 2008; Muetzel et al. 2008; Lebel and Beaulieu 2011). For the majority of WM tracts, childhood development consisted of a relatively steep increase in FA and decrease in MD, eventually plateauing in early adolescence or adulthood (Lebel and Beaulieu 2011). A large cross-sectional study suggested that peak FA is reached at a mean age of 30 years (Imperati et al. 2011), whereas longitudinal studies suggest that peak FA may occur between 20 and 42 years of age (Lebel et al. 2012). However, different WM tracts may have slightly different

developmental trajectories. For example, fronto-temporal connections, including the cingulum, the superior longitudinal fasciculus, and the uncinate fasciculus, appear to have more prolonged maturation (Lebel et al. 2012). During adulthood, between the ages of 18 and 72 years of age, radial diffusivity (RD), which is thought to be most influenced by myelination, appeared to be stable in most of the WM (Wu et al. 2011). In contrast, axial diffusivity (AD), which is most affected by properties of axons, appeared to decrease with age (Wu et al. 2011).

As mentioned above, it is possible that rapid early brain development that occurs in some very young children with autism may be developmentally related to the increased FA found in some very young children autism (Ben Bashat et al. 2007; Weinstein et al. 2011; Wolff et al. 2012). In cross-sectional studies with older samples, differential correlations between DTI measures and age in persons with ASD compared to persons with typical development also suggest atypical development of WM microstructure in ASD. Specifically, significant age-by-diagnosis interactions have been found across multiple cross-sectional studies, suggesting that age and FA are positively related in persons with typical development more so that in persons with ASD in areas such as the superior temporal gyrus WM (Lee et al. 2007) and the WM tracts of the frontal lobe (Cheng et al. 2010). However, this has not consistently been the case, as other studies have demonstrated similar age-related changes in FA or MD of both groups (Pugliese et al. 2009; Bode et al. 2011), or even increased FA with age in ASD (Keller et al. 2007). As ASD is a developmental disorder, future longitudinal research is needed to determine if WM microstructure develops similarly with age in persons with ASD compared to persons with typical development.

8.4.3 Whole-Brain DTI Findings Across Development in ASD

Several studies have examined WM across the brain in older children, adolescents, and adults with ASD to measure the integrity of whole-brain WM or determine where in the brain significant case–control differences in measures of WM integrity exist. The whole-brain analyses have consistently demonstrated reduced FA and increased MD in ASD (Travers et al. 2012). Nevertheless, studies using ROI or voxel-based approaches have found widespread, but not uniformly diffuse, areas of WM in which individuals with ASD have decreased FA compared to individuals with typical development. Jou et al. (2011a) found decreased FA in children and adolescents in WM tracts that connect brain areas associated with social functioning, even after controlling for age and IQ. Bloemen et al. (2010) found decreased FA and increased RD across a number of different areas of the brain in adults (25–52 years old) with Asperger's syndrome. Similarly, Lee et al. (2009) used a novel voxel-based method and found that MD was significantly increased for almost all the WM, though FA differences were more localized. These studies generally demonstrate that children and adults with ASD are likely to have reduced FA and increased MD and RD compared to individuals with typical development, and these studies confirm that widespread areas of the brain are affected. Next, we discuss some of the specific brain regions that may be particularly affected in ASD.

8.4.4 DTI Findings in the Frontal Lobe Across Development in ASD

The frontal lobe has been of high interest in autism because frontal WM, particularly prefrontal WM, may contribute to the early brain overgrowth seen in some children with autism (Herbert et al. 2004; Courchesne et al. 2011). Several functional imaging studies have found evidence of functional underconnectivity WM tracts connecting frontal lobe regions with posterior parts of the brain (Schipul et al. 2011). A recent preliminary postmortem study of the prefrontal cortex provides evidence of developmental pathology in the frontal lobe in ASD (Courchesne et al. 2011; Lainhart and Lange 2011).

Whole-brain voxel-based studies, which include WM tracts and gyral WM, have found inconsistent evidence of microstructural abnormalities in frontal lobe WM in ASD. Compare, for example, the study of Lee et al. (2009) with the study of Noriuchi et al. (2010).

One research group has specifically targeted the frontal lobe for a detailed examination of its WM using DTI. Table 8.2 summarizes the results. In the first study, tractography was used to measure microstructural parameters and the distribution of fiber lengths. The frontal lobe was divided into two large WM compartments in each hemisphere, the peripheral WM compartment, close to the cortex, where short fibers are located (in addition to the terminal ends of long-range fibers), and the central WM, comprising long-range association, commissural, and projection fibers (Sundaram et al. 2008). Fifty children with ASD were compared to 16 typically developing children. The hypotheses tested were greater microstructural abnormality in the central compartment (long-range fibers) than in the peripheral compartment (short-range fibers) and increased number of short-range fibers. The results did not support the hypotheses. FA was decreased, and apparent diffusion coefficient (ADC; a measure similar to mean diffusivity) was increased in the short-range fiber compartment in both left and right frontal lobes of the ASD group. In the long fiber compartment, average ADC was increased bilaterally but no case–control differences in FA were found. Examination of the distribution of fiber lengths showed no evidence of ASD-control differences in the number of short-range fibers or long-range fibers measured by tractography, but there was evidence of increased length of the long fibers in the frontal lobe in the ASD group.

In the second study (Kumar et al. 2010), the investigators used tractography to measure mean FA and ADC in frontal lobe tracts of interest (i.e., long-range fibers running between the frontal lobe and other parts of the brain). The bilateral uncinate fasciculi, inferior fronto-occipital fasciculi, arcuate fasciculi, cingulum bundle, corticospinal tract, and corpus callosum were examined. The results showed a decrease in mean FA in the right uncinate fasciculus, left arcuate fasciculus, right cingulum, and corpus callosum. Inclusion of a non-autistic developmentally delayed (DD) comparison group in addition to a typically developing control group allowed the investigators to determine that only the decrease in FA in the left arcuate fasciculus was specific to autism; the decrease in FA found in the other tracts in the ASD group was also found in the DD group. No increase in ADC specific to autism was

Table 8.2 Summary of results from a set of studies examining frontal lobe WM and frontal lobe tracts in ASD (Sundaram et al. 2008; Kumar et al. 2010; Jeong et al. 2011)

WM area of interest	Differences in the ASD sample		
Frontal lobe peripheral WM compartment (short-range fibers and terminations of long-range fibers)	Decreased FA		Increased ADC
Frontal lobe central WM compartment (long-range association, commissural, and projection fibers)	No difference in FA		Increased ADC
Long-range tracts (to and from the frontal lobes, including the parts of the tracts in and outside of the frontal lobe)	Abnormalities specific to ASD Decreased FA: Lt arcuate fasciculus Increased fiber length, volume, and density: Rt uncinate fasciculus Increased fiber length: Lt cingulate bundle Increased fiber length and density: corpus callosum	Abnormalities shared by ASD and DD Decreased FA: Rt uncinate fasciculus, Rt cingulate bundle, genu CC Decreased fiber length: Lt uncinate fasciculus	No difference in ADC specific to ASD Increase in ADC found in both ASD and DD groups: Right arcuate fasciculus
	Tract curvature Increased curvature: bilateral arcuate fasciculus Bilateral uncinate fasciculus Genu of the corpus callosum		

observed in any of the tracts. The only nonspecific increase in ADC found in both ASD and DD groups was in the right arcuate fasciculus. Voxel-based analysis of skeletons of the tracts using tract-based spatial statistics showed similar results. Other abnormalities specific to autism were increased fiber length, volume, and density in the right uncinate, increased fiber density in the left cingulum, and increased fiber length and density in the corpus callosum.

In the third study, tract-based morphometry was used to measure the macrostructural curvature of the tracts (Jeong et al. 2011). Curvature was increased in the arcuate fasciculus and uncinate fasciculus in both hemispheres and in the genu of the corpus callosum. The arcuate fasciculus bent more at the parieto-temporal junction. The uncinate fasciculus bent more at the frontotemporal junction, and the genu bent more at the midline in the ASD group compared to typically developing controls. FA was decreased and radial diffusivity was increased at the bending regions of the tracts in the ASD group. The authors propose that the findings are consistent with thinner and more numerous axons in the affected tracts, which would slow velocity of conduction, break down language functioning, and result in functional underconnectivity in individual with ASD.

The combined results suggest an abnormality of WM microstructural integrity/organization in the WM just below the cortex of the frontal lobe, a more generalized

abnormality of ADC in the frontal lobe affecting both peripheral and central WM; abnormal integrity of the left arcuate fasciculus specific to autism; nonspecific abnormalities of the integrity of several other tracts; specific and nonspecific changes in fiber tract length, volume, and/or density; and abnormal curvature with excessive bending of the arcuate fasciculus and uncinate fasciculus bilaterally and the genu of the corpus callosum. To what extent the findings represent generalized phenomena in ASD is not known. The FA and ADC findings differ from some studies using voxel-based approaches in older samples, and many of the other results have not yet been replicated.

The set of results, nevertheless, shows the value of systematic examination of regions of the brain using different image analysis methods to examine different facets of potential pathology.

8.4.5 DTI Findings in the Arcuate Fasciculus Across the Development in ASD

As mentioned previously, the arcuate fasciculus is a long intrahemispheric WM tract that connects the frontal lobe to the temporal and parietal lobes. The arcuate fasciculus has been long known to be an essential WM pathway for language understanding and production. For example, Catani and colleagues (2005) found that the arcuate fasciculus enables communication between Broca's area (inferior frontal gyrus) and Wernicke's area (superior temporal sulcus) through a direct pathway and an indirect pathway via Geschwind's area (inferior parietal lobule). The results examining the arcuate fasciculus as frontal WM tract are discussed in the previous section. However, the long-range connectivity of this tract and its contribution to language ability warrant a more in-depth review of the arcuate fasciculus's WM microstructure in individuals with ASD.

Table 8.3 summarizes studies that have specifically examined the arcuate fasciculus. Across these studies, arcuate fasciculus FA was decreased in younger, cognitively lower functioning, and more language-impaired ASD children (Kumar et al. 2010; Lai et al. 2012) but not in higher-functioning individuals (Fletcher et al. 2010; Knaus et al. 2010). MD/ADC was increased in the arcuate fasciculus in the two studies that measured it. In higher-functioning children, MD was significantly increased compared to typically developing children (Fletcher et al. 2010). In younger and lower functioning children, FA was similarly decreased in the ASD children and children with non-autistic developmental delay compared to typically developing children (Kumar et al. 2010). The latter finding suggested that in younger, low-functioning children with ASD, increased MD in the arcuate fasciculus is not specific to autism. Two studies, one in high-functioning young people with ASD and the other in lower-functioning children, found increased activation of the left inferior frontal gyrus associated with increased FA in the arcuate fasciculus (Knaus et al. 2010; Lai et al. 2012), suggesting a relationship between functional activation and structural connectivity of the arcuate fasciculus.

Some children with autism do not develop spoken language; they remain nonverbal often for their entire lives. The neural basis of this profound defect is unknown. In the first small preliminary study to exclusively examine nonverbal children with autism, 4 of 5

Table 8.3 Case–control results of ROI DTI studies of the arcuate fasciculus

Reference	Fletcher et al. (2010)	Knaus et al. (2010)	Kumar et al. (2010)	Lai et al. (2012)	Wan et al. (2012)	Lewis et al. (2012)
Mean age (years)	14.2	16.1	5.0	11.0	6.7	
Age range (years)	11–17	11–19	2.5–8.0	5.8–17.8	5.8–8.8	0.5–25
Groups compared and sample sizes	0 HFA males 10 TD males	14 HF ASD males with delayed onset language 17 TD males	32 ASD (29 males) 16 TD (12 males) 12 DD (10 males)	16 LFA (14 males) most with limited verbal ability 18 TD	5 ASD completely nonverbal 5 TD	12 ASD+TSC (i.e., non-idiopathic ASD) 30 TSC only 45 TD
Tesla	3.0	3.0	3.0	1.5	3.0	3.0
b-value (s/mm^2)	1,000	1,000	1,000	1,000	1,000	1,000
Imaging analysis type	New volumetric DTI segmentation of AF followed by analysis of FA, MD, RD, and AD	Tractography of AF using fMRI-identified seed regions, followed by analysis of mean FA	Whole-brain tractography used for DTI segmentation of AF followed by analysis of FA, ADC, and fiber characteristics; also TBSS to determine voxels with significant ASD—control differences skeletonized tracts	AF tractography from Heschl's gyrus seed to inferior frontal gyrus—ventral and dorsal AF tracts. FA, MD, and orthogonal tensor norm values measured, as well as quantified tract termination location	AF tractography from posterior temporal lobe and temporal-parietal junction to inferior frontal gyrus. AF volume measured	AF tractography using specified ROIs: WM adjacent to Broca's area, Geschwind's territory, and Wernicke's area. FA and MD measured
Results						
	MD significantly increased in the autism group, mainly due to increased RD, specific to the AF and not due to brain-wide changes; FA and MD were less lateralized in the autism group	ASD vs TD: no differences in mean FA; in both ASD and TD, subjects with greater Lt. lateralization of fMRI language activation had higher FA of AF	ASD vs TD: Mean FA lower in Lt. AF (specific to ASD—not found in DD) Mean ADC higher in Rt. AF (but nonspecific to ASD—also found in DD) No difference in average fiber length, density, or volume	LFA vs TD Mean FA in left dorsal tract lower in autism No FA differences in left or right ventral tract or Rt dorsal tract; no differences in MD Lt. AF FA correlated with fMRI activation in Lt. IFG for speech and song	Nonverbal ASD vs TD: all TD children had the expected Lt. > Rt. volumetric asymmetry of AF but 4 of 5 of the nonverbal ASD children did not	TSC had lower FA and higher MD in AF than TD; TSC+ASD had even lower FA and higher MD in AF

ASD autism spectrum disorder, *HFA* high-functioning autism (with performance IQ > 70), *LFA* low-functioning autism, *TSC* tuberous sclerosis complex, *AF* arcuate fasciculus, *IFG* inferior frontal gyrus, *TD* typically developing, *DD* non-autistic developmental delay, *FA* fractional anisotropy, *MD* mean diffusivity, *RD* radial diffusivity, *AD* axial diffusivity, *ADC* apparent diffusion coefficient (similar to MD), *TBSS* tract-based spatial statistics (using tract skeleton)

(80 %) had loss of the typical left > right volumetric asymmetry of the arcuate fasciculus, which was normally present in 5 of 5 typically developing controls (Wan et al. 2012).

One DTI investigation was performed in children with syndromic autism. Autism is idiopathic, i.e., without a known likely causal factor in up to 90 % of cases. In other cases, referred to as syndromic autism, the ASD occurs in the context of a genetic condition, such as tuberous sclerosis complex. Lewis et al. (2012) examined WM microstructure in the arcuate fasciculus in two subgroups of children with TSC. One of the subgroups had ASD in addition to TSC, and the other subgroup did not. The severity of changes in FA and MD in the arcuate fasciculus was related to the co-occurrence of autism. Although FA was decreased and MD was increased in the arcuate fasciculus of both TSC subgroups, the abnormalities were greater in the subgroup with ASD. Because the majority of patients with tuberous sclerosis are intellectually handicapped, this finding suggests, in comparison to Kumar et al. (2010), that while WM microstructural abnormalities of the arcuate fasciculus might be present in developmentally impaired individuals in general, the abnormalities may be specifically more severe when autism is also present.

It is important to note that the arcuate fasciculus is a subsection of the larger superior longitudinal fasciculus. In addition to the arcuate fasciculus studies mentioned above, a number of studies have investigated the WM microstructure of the broader superior longitudinal fasciculus in persons with ASD. However, as reviewed by Travers et al. (2012), the results of these studies have been quite inconsistent. The lack of consistency of these results may be caused in part by how long and complex the superior longitudinal fasciculus is, making it difficult to trace. It is also possible that differences in participant characteristics such as age, language ability, verbal IQ, or ASD communication symptom severity may account for some of the inconsistencies. However, Travers and colleagues (2012) examined if average reported IQ measures or age of the ASD group in these studies accounted for the differences in findings and found that most of the studies used similar ages and IQ groups (with the exception of Jeong et al.'s and Weinstein et al.'s studies that used a younger age group, and Lai et al. and Wan et al. who examined lower-functioning and nonverbal children). This suggests that at the group level these studies were similar in these characteristics. However, the microstructure of the arcuate fasciculus/superior longitudinal fasciculus has not been directly compared in cognitively low- and high-functioning subgroups or in verbal and nonverbal subgroups. Therefore, examining heterogeneity in these regions as a function of characteristics of the individual participants may be a critical avenue for future research. Nagae et al. (2012) used region-of-interest tractography to examine FA and MD in the superior longitudinal fasciculus (SLF) and temporal part of the SLF (tSLF, which is the temporal lobe portion of the arcuate fasciculus) in 35 children with ASD compared to typical controls. The ASD children were in two subgroups, one with language impairment and the other without language impairment. Language impairment was defined as a standard score <85 on the CELF-4 Core Language Index. Although there was not a difference in FA between the ASD subgroup and typically developing controls, there were significant MD differences. Specifically, MD was increased in the left SLF

in the combined ASD group. The increase in MD was mainly due to greater MD in the tSLF and in the subgroup of ASD children with significant language impairment. These results provide preliminary evidence that individual differences in language functioning may account for some of the inconsistent results in the literature regarding the WM integrity of the arcuate fasciculus/SLF in ASD. Further, this finding by Nagae and colleagues (2012) is consistent with other studies that have found decreased FA in ASD in temporal lobe aspects of this tract (Barnea-Goraly et al. 2004, 2010; Lee et al. 2007; Noriuchi et al. 2010); for an exception to this finding, see Cheng et al. (2010).

8.4.6 DTI Findings in the Uncinate Fasciculus Across the Development in ASD

The uncinate fasciculus is a hook-shaped tract that is known to directly connect medial temporal areas with frontal cortices. Studies in individuals with ASD have found inconsistent evidence for group differences in DTI measures of this tract. As mentioned above, infants who developed ASD had increased mean FA in the uncinate fasciculus at 6 months of age but not at 12 or 24 months of age (Wolff et al. 2012). In older individuals, in the bilateral uncinate fasciculus, research has found increased FA (Sahyoun et al. 2010a), decreased FA (Cheon et al. 2011; Jeong et al. 2011; Poustka et al. 2012), increased MD and RD (but no difference in FA) (Ameis et al. 2011; Shukla et al. 2011a), or no FA or MD group difference (Pugliese et al. 2009). As with the arcuate fasciculus, future analyses of the uncinate fasciculus should account for individual characteristics of the sample to possibly clarify these results.

8.4.7 Atypical Lateralization of WM Across Development in ASD

Most research has found a leftward asymmetry in DTI measures in the arcuate fasciculus and uncinate fasciculus in persons with typical development (e.g., greater FA and less MD in the left hemisphere) (Catani et al. 2007; Hasan et al. 2009). However, this pattern of lateralization may not be as prevalent in persons with ASD. Indeed, multiple studies of individuals with ASD have found decreased leftward lateralization in ASD in the arcuate fasciculus (Fletcher et al. 2010; Lo et al. 2011), in the uncinate fasciculus (Lo et al. 2011), and in the WM of the superior temporal gyrus (Lange et al. 2010). However, a study by Knaus et al. (2010) suggests that atypical lateralization of the arcuate fasciculus may not be specific to ASD, but ASD may make it more likely for individuals to be less typically lateralized. This lack of lateralization may be related to decreased language functioning (CELF-3) (Fletcher et al. 2010), and as mentioned above (Wan et al. 2012), loss of normal asymmetry of the arcuate fasciculus may be particularly prominent in children with ASD who are nonverbal.

8.4.8 DTI Findings in Midline WM Tracts Across Development in ASD

The corpus callosum and cingulum are the WM tracts that run along the midline of the brain. The corpus callosum is the major bundle of WM tracts that connects the left and right hemispheres and contributes to interhemispheric connectivity. The cingulum bundles run above the corpus callosum from the anterior cingulate cortex to the posterior cingulate area and are the primary intrahemispheric association pathways for the medial cingulate cortex and temporal lobe structures. Both the corpus callosum and cingulum make important contributions to cognitive functions that may be implicated in ASD. For example, interhemispheric connectivity through the corpus callosum is vital to a number of cognitive functions, and functional imaging studies show that as receptive language complexity increases, from the comprehension of single words to sentences, to narrative, interhemispheric functioning appears to be increasingly involved (Xu et al. 2005). Similarly, the cingulum is functionally involved in attention, response inhibition, emotional behavior, and the processing of pain (Lezak et al. 2012). Anterior and posterior portions of the cingulum (with recent evidence suggesting four portions) have different projections and roles (Vogt 2005).

The corpus callosum and cingulum appear to be important brain regions of interest for the study of ASD. In terms of the corpus callosum, multiple studies have found decreased cross-sectional area or volume in ASD (Hardan et al. 2000; Vidal et al. 2006; Alexander et al. 2007; Kilian et al. 2008; Keary et al. 2009; Thomas et al. 2011; DuBray Prigge et al. 2012). In addition, Anderson and colleagues (2011) found decreased interhemispheric functional connectivity in ASD. In terms of the cingulum, Pugliese et al. (2009) found an increased number of streamlines in the bilateral cingulum in persons with Asperger's syndrome.

As reviewed in Travers et al. (2012), a number of DTI studies have found significantly decreased FA and increased MD and RD in ASD across the entire corpus callosum (i.e., genu, body, and splenium) (Table 8.4) and in the anterior cingulum (Barnea-Goraly et al. 2004; Thakkar et al. 2008; Lee et al. 2009; Pardini et al. 2009; Kumar et al. 2010; Noriuchi et al. 2010; Jou et al. 2011a, b). However, these results have not been entirely consistent. In addition to the studies using quite young subjects that found increased FA in the corpus callosum of children with ASD (Ben Bashat et al. 2007; Weinstein et al. 2011; Wolff et al. 2012), other studies with older participants have failed to find group differences in corpus callosal FA (Cheung et al. 2009; Cheng et al. 2010; Hong et al. 2011) or in the cingulum FA (Cheng et al. 2010).

Although most of the corpus callosum studies have demonstrated decreased FA in persons with ASD, one possible explanation for the inconsistent results is sample heterogeneity. Specifically, Alexander and colleagues (Alexander et al. 2007) found that the ASD-control group difference in corpus callosum FA was driven by a subgroup of 28 % of participants with ASD who had low FA of the corpus callosum (compared to the 72 % of ASD participants who demonstrated typical FA in this region). This subgroup also exhibited decreased performance IQ, increased MD, increased RD, and decreased corpus callosum volume compared to their ASD peers. This ASD subgroup finding highlights the importance of examining individual variability within the ASD

Table 8.4 Results of DTI studies investigating FA of the corpus callosum

Study	ASD *N*	TD *N*	Age range (years)	FA corpus callosum findings in ASD
Alexander et al. (2007)	43	34	7–33	Decreased FA
Barnea-Goraly et al. (2004)	7	9	14.6 ± 3.4	Decreased FA
Barnea-Goraly et al. (2010)	13	11	6–13	Decreased FA
Brito et al. (2009)	8	8	6–12	Decreased FA
Jou et al. (2011b)	10	10	8–19	Decreased FA
Kumar et al. (2010)	32	16	2.5–8.9	Decreased FA
Noriuchi et al. (2010)	7	7	11–18	Decreased FA
Shukla et al. (2010)	26	24	9–18	Decreased FA
Shukla et al. (2011a)	26	24	9–20	Decreased FA
Ben Bashat et al. (2007)	7	18	1.8–3.3	Increased FA
Weinstein et al. (2011)	21	26	1.5–5.8	Increased FA
Hong et al. (2011)	18	16	8.7 ± 2.18	No difference in FA
Cheng et al. (2010)	25	25	13.71 ± 2.54	No difference in FA
Cheung et al. (2009)	13	14	6–14	No difference in FA
Wolff et al. (2012)	28	64	0.5–2	Increased FA at 6 months, decreased FA at 24 months

group when examining group differences in WM integrity, and this suggests that some but not all persons with ASD have decreased FA in the corpus callosum.

8.4.9 DTI Findings in the Hippocampal/Amygdala Region and Pathways Across the Development in ASD

Some voxel-based DTI studies of autism have found significant ASD-control differences in the WM around the amygdala, in addition to other areas (Barnea-Goraly et al. 2004; Noriuchi et al. 2010).

Conturo et al. (2008) examined macrostructure and microstructure of the bidirectional pathways between the amygdala and fusiform gyrus (A–F) and the hippocampus and fusiform gyrus (H–F). Cognitively high-functioning adolescents and adults with autism were compared to pairwise-matched typically developing controls. Macrostructure of the A–F and H–F fiber pathways, including pathway shapes, trajectories, and location of terminations, appeared normal in the autism group. The right hippocampal–fusiform pathway had *decreased* diffusion perpendicular to the fiber tract, and the decrease correlated with more impaired performance on the Benton Face Recognition task. The decrease in diffusion in the H–F pathway appeared specific with the brain; it remained significant when normalized for whole-brain perpendicular diffusion. Decrease in diffusion perpendicular to the right H–F fiber pathway in the autism group resulted in the loss of the typical lateralization of the H–F pathway seen in the controls.

The right H–F fiber pathway finding is interesting because it is in the opposite direction to the change in radial diffusivity, an increase in perpendicular diffusion, found most commonly in autism. The correlation with more impaired face

recognition suggests that the finding is not spurious but has functional consequences. In contract to the specific decrease in perpendicular diffusion in the right H–F pathway, statistical trends for nonspecific increases in perpendicular diffusion were found in the left H–F pathway and bilaterally in the A–F pathways; the latter changes appeared, in part, due to increased whole-brain perpendicular diffusion.

This study by Conturo et al. (2008) is a noteworthy example of how measuring neuropsychological functioning specific to a particular WM pathway can inform the results of DTI studies. Along with Fletcher et al. (2010), the report also shows the scientific value of determining if case–control differences in tensor summary measures of a particular pathway are specific to the pathway or part of a more global brain process. This study also exemplifies how integration of neuropsychological and DTI findings with result of postmortem studies, knowledge of neural functioning, and careful and systematic consideration of alternative mechanisms enhances understanding of the possible meaning of the results.

A fascinating multimodal imaging study of a 63-year-old man with autism accompanied by multiple savant skills (his most outstanding ability in art) examined volumes and WM microstructure parameters (Corrigan et al. 2012). Regions of interest were the corpus callosum; WM in the regions of the amygdala, hippocampus, and caudate nucleus; and frontal and occipital lobe WM. Significant differences, greater than 2 standard deviations from the means of 7 highly educated adult males without autism and savant skills, were found in the amygdala and hippocampal regions, corpus callosum, and occipital lobe. Fiber tract bundle volumes were larger in the right than in left hemisphere in the medial temporal lobe area. FA was decreased in the left hippocampal region by more than 2 standard deviations. In both left and right hippocampal regions, mean diffusivity was increased by more than 3 standard deviations, axial diffusivity was increased by 8 standard deviations, and radial diffusivity was increased by 2–3 standard deviations above the control mean. In the left amygdala, mean diffusivity, axial diffusivity, and radial diffusivity exceeded the control means by more than 3 standard deviations. The results suggest that consideration of individual differences in ability and disability is important in interpreting and understanding WM microstructural changes in autism.

8.4.10 DTI Findings in the Thalamic Radiations Across the Development in ASD

A key area in sensory processing, the connections of the thalamus, is also of interest in the study of autism. Table 8.5 summarizes DTI studies reporting results for the thalamic radiations. Case–control differences have been found most consistently in the anterior thalamic radiations, which project to rostral prefrontal cortex. Microstructural changes in the anterior thalamic radiations (decreased FA and in one study increased MD and RD) have been found in ASD individuals at 12 months of age, 24 months of age, and in older ASD samples at mean ages 11, 12.8, and 39 years. The results, which need to be verified by longitudinal studies in older children and adults with ASD, suggest continuity of a tendency toward microstructural changes in

Table 8.5 Case–control comparisons of DTI results in the thalamic radiations

	Wolff et al. (2012)	Lee et al. (2009)	Cheung et al. (2009)	Bloemen et al. (2010)	Cheon et al. (2011)	Shukla et al. (2011a)
Mean age ASD	6, 12, and 24 months of age	16.2 years	9.3 years	39 years	11.0 years	12.8 years
Age range		7–33 years	6–14 years	23–54 years	8–14 years	9–20 years
Groups compared and sample sizes	28 high-risk infants who developed autism 64 high-risk infants who did not develop autism	43 HF ASD males (38 autism) 34 TD males	13 HF autism (12 males) 14 TD (13 males)	13 Asperger's males 13 TD males	17 ASD males 17 TD males	26 HF ASD (15 autism; 25 males) 24 TD
Tesla	3.0	3.0	1.5	1.5	1.5	3.0
b-value (s/mm^2)	1,000	1,000	1,200	1,300	900	2,000
Imaging analysis type	ROI tractography to measure mean FA of anterior thalamic radiations	Novel voxel-based whole-brain analysis of FA, MD, AD, and RD	Voxel-based whole-brain WM analysis of FA maps	Whole-brain voxel-based analysis of FA, MD, and RD	TBSS for ROI voxel-based analysis of FA, MD, AD, RD for thalamic radiations, and 3 other tracts	TBSS for whole-brain voxel-based analysis of FA, MD, AD, and RD
Results						
	High-risk infants who developed autism had no difference from comparison infants in FA at age 6 months, but FA was lower at 12 and 24 months	FA decreased and MD, AD, and RD increased in Lt. posterior thalamic radiations; MD increased in Rt. posterior thalamic radiations	No FA differences in thalamic radiations	FA decreased in parts of the Lt. anterior thalamic radiations; DR increased possibly in the posterior and superior thalamic radiations	FA decreased in anterior thalamic radiations bilaterally, but not in superior or posterior thalamic radiations	FA decreased and MD and RD increased in the anterior thalamic radiations

the anterior thalamic radiations from one year of age into adulthood. Differences have also been found in the posterior thalamic radiations that project to the occipital cortex and in one study possible differences in the superior thalamic radiations.

8.4.11 DTI Findings in the Cerebellar WM Across the Development in ASD

Catani et al. (2008) performed careful tractography to "dissect" and analyze short intracerebellar fiber connections and long afferent and efferent cerebellar tracts running into and out of the cerebellum, respectively. Adults with Asperger's syndrome were found to have decreased FA in the short intracerebellar fiber connection compartment (likely purkinje cell and granule cell parallel fibers) and in the superior cerebellar peduncle tract (long efferent fibers). No differences in FA were found in the two long afferent tracts running into the cerebellum via the inferior and middle cerebellar peduncles. In addition, case–control differences in MD were not found in the any of the fiber tracts examined. The results suggest specific pathology in the cerebellum in the Asperger's sample. Abnormalities in the short intracerebellar fiber connections and long efferent tracts could neurologically impair the fine-tuning of motor movements, motor learning, and other non-motor functions. The results are important because many individuals with Asperger's syndrome and other ASDs have motor impairments and the neural cause is unknown. A follow-up whole-brain voxel-based DTI analysis by the same group of investigators, however, did not replicate the case–control differences in FA (Bloemen et al. 2010).

Table 8.6 summarizes studies that have examined cerebellar fiber connections and tracts. It is apparent that WM microstructural studies of the cerebellum in ASD are few in number.

8.4.12 DTI Measures of WM in Males and Females with ASD

Studies in typically developing individuals have examined differences between males and females in indices of WM microstructure. The results, reviewed in Wu et al. (2011) and Kanaan et al. (2012), are inconsistent. The latter study compared 45 women to 90 men, mean ages 24 (standard deviation 7.9) and 25 (standard deviation 6.1) years, respectively, matching the groups for age, handedness, years of education, and intelligence. Using a voxel-based approach (confirmed by ROI analysis when indicated by possible partial volume influences), the investigators found that the female sample had higher FA in the corpus callosum, whereas the male sample had higher FA in the anterior portion of the left superior longitudinal fasciculus and cerebellum. The differences between the males and females were larger than differences found between patients and controls in studies of patients with developmental neuropsychiatric disorders, such as schizophrenia. The authors raised the concern

Table 8.6 Case–control comparisons of DTI results in the cerebellum

Reference	Catani et al. (2008)	Bloemen et al. (2010)	Sivaswamy et al. (2010)	Shukla et al. (2010)
Mean age	31 years	39 years	5.0 years	12.7 years
Age range	18–49 years	23–54 years	2.6–9 years	9–18 years
Groups compared and sample sizes	15 Asperger males 16 TD males	13 Asperger males 13 TD males	27 autism (24males) 16 TD	26 ASD (15 autism, 11 Asperger; 24males) 24 TD (23 males)
Tesla	1.5	1.5	3.0	3.0
b-value (s/mm^2)	1,300	1,300	1,000	2,000
Image analysis type	2 ROI approach for deterministic tractography for measure of FA and MD in the short intracerebellar fibers and long afferent (middle and inferior cerebellar peduncle) and efferent (superior cerebellar peduncle) tracts	Whole-brain voxel-based analysis of FA, MD, and RD	ROIs drawn to measure FA and MD in superior, middle, and inferior cerebellar peduncle tracts	ROIs drawn in native space for analysis of FA, MD, AD, RD in the middle cerebellar peduncle.
Results				
	FA decreased in Rt. superior cerebellar peduncle tract (long projection fibers) and right short intracerebellar fibers No difference in MD	No difference in FA in the cerebellum	No differences in FA in any of the tracts, but FA asymmetry in middle and inferior cerebellar peduncles seen in TD was reversed in autism MD was increased in bilateral superior cerebellar peduncles in the autism group	FA decreased in middle cerebellar peduncle; no difference in MD, AD, and RD FA decreased and MD and RD increased in the whole brain

that an imbalance between the male-to-female ratio in cases and controls, which is common in neuropsychiatric studies, could confound the results. In contrast, Wu et al. (2011), using hybrid diffusion imaging (HYDI) and three different types of image analysis (whole-brain, ROI, and voxel-based), found little effect of gender on measures of WM microstructure in adults 18 years of age and older.

A preliminary study of 28 adults with Asperger's syndrome (15 males, 13 females) and 30 healthy control adults (15 males, 15 females) examined sex × diagnosis interactions in FA and MD in subregions of the corpus callosum and 4 bilateral ROIs (Beacher et al. 2012). Differences in WM microstructure between healthy males and females in this study were different from the findings of Kanaan et al. (2012). The sexual-dimorphic findings in the typically developing adults in the Beacher et al.'s (2012) study were less than or absent in the adults with Asperger's syndrome. The investigators suggest that autism may attenuate typical male–female differences in brain WM microstructure.

It is apparent from these studies that much remains to be learned about sexual dimorphism of WM microstructure in both the typically developing brain and the brain affected by ASD. The results nevertheless highlight the importance of careful consideration of potential sex effects and diagnosis × sex interactions in studies of WM microstructure in ASD.

8.4.13 ASD Subtypes, Differences in Clinical Features, and DTI Measures of WM

Given that WM microstructure has been frequently found to be atypical in persons with ASD, it is essential to examine how WM microstructure may be related to the behavioral and cognitive symptoms of ASD. As reviewed by Travers et al. (2012), there are a number of studies that have tried to link DTI measures to cognition and ASD symptom severity. However, these relations have been quite unclear and inconsistent, possibly due to the small sample sizes in these studies. Some studies have failed to find relations between symptom severity and DTI measures (Sundaram et al. 2008; Barnea-Goraly et al. 2010; Shukla et al. 2010; Hong et al. 2011; Langen et al. 2012), whereas other studies have found decreased FA associated with increased symptom severity (Catani et al. 2008; Thakkar et al. 2008; Cheung et al. 2009; Noriuchi et al. 2010) or even decreased FA associated with decreased symptom severity in ASD (Ke et al. 2009). Further work with larger sample sizes is definitely needed to try to understand the behavioral manifestations of decreased WM microstructure.

8.4.14 Demographic and Clinical Factors to Consider in DTI Measures of WM

As discussed above, WM microstructure changes with age, from birth to late adulthood. The changes are characterized by increases in diffusion tensor anisotropy and decreases

in the MD during childhood (Huppi et al. 1998; Mukherjee et al. 2001; O'Sullivan et al. 2001; Schmithorst et al. 2001), followed by a gradual decrease in anisotropy in adulthood (Pfefferbaum et al. 2000; Abe et al. 2002). It is absolutely critical to control for the effects of age in quantitative DTI studies. In addition, one cannot assume the relationship between age and WM microstructure is the same in typically developing controls and individuals with a brain disorder such as autism or schizophrenia (Jones et al. 2006). Potentially different relationships between age and DTI measures in case and controls need to be directly examined by testing for age × group interactions. Lack of awareness of a significant age × group interaction can significantly affect the interpretation of study results (Jones et al. 2006). Sexually dimorphic effects on WM microstructure and sex × group interactions, discussed above, are also critical to consider.

Other concerns in DTI studies of autism include psychotropic medication use and sedation for scanning. These issues have been statistically considered at the group level of analysis using psychotropic medication use as a dichotomous variable, with "use" referring to use of any type or multiple types of psychotropic medications. To date, no major effects in the direction of increasing case–control differences have been found (Alexander et al. 2007; Lee et al. 2007). Use of sedation for scanning, which is more often needed in younger and lower-functioning individuals with autism and not used in typically developing controls, is another issue. Once again, to date no major effects of the use of propofol for DTI scanning have been found (Alexander et al. 2007; Lee et al. 2007).

Another concern is "comorbidity." Many individuals with autism meet criteria for psychiatric conditions in addition to having the core diagnostic features of ASD (Leyfer et al. 2006; Mazefsky et al. 2012). Community diagnoses in children and adults with ASD referred to research studies may be much different than diagnoses made using standardized measures (Mazefsky et al. 2012). About one-third of individuals with autism develop seizures at some time during development (Tuchman and Cuccaro 2011). Specific non-ASD psychiatric disorders and specific types of seizures may be associated with specific DTI findings even in the absence of differences in WM volume. As a result, these conditions must be considered in DTI studies of ASD. Some investigators exclude participants with ASD who are taking psychotropic medications or who have any "comorbid" psychiatric conditions. This exclusion results in samples that are not representative of the population of ASD individuals. Other research teams measure the comorbidity and consider it in the statistical analysis of the data.

8.5 Summary of Findings

In the last eight years, a surprising number of studies have in vivo examined WM microstructure in the brains of persons with ASD using DTI methodology. The results of the majority, but not all, of these studies have indicated that WM integrity in ASD may be atypical (Table 8.7). Specifically, it appears that individuals with ASD often have decreased FA and increased MD and RD, but this pattern may be more relevant to some WM tracts compared to others. Of the tracts examined in this review, there was strong evidence for group differences in WM of the corpus

Table 8.7 Summary of all DTI published studies of ASD

Reference	ASD sample size (*N*)	TD sample size (*N*)	ASD age in years (mean ± SD)	Control age in years (mean ± SD)	ASD IQ (mean ± SD)	Control IQ (mean ± SD)	DTI findings	DTI correlates
Barnea-Goraly et al. (2004)	7	9	14.6 ± 3.4	13.4 ± 2.8	101 ± 12.2	107 ± 8.5	Reduced FA in the ASD near ventral prefrontal cortex, ACC, TPJ, STS, occipitotemporal tracts, and corpus callosum	–
Alexander et al. (2007)	43	34	16.2 ± 6.7	16.4 ± 6.0	107.5 ± 13.0	112.8 ± 12.1	Reduced FA in ASD in the corpus callosum (with increased MD and RD)	FA and RD of corpus callosum correlated with age (in both groups) and with PIQ in ASD (not control). Corpus callosum MD and genu FA correlated with processing speed in ASD
Ben Bashat et al. (2007)	7	18	1.8–3.3	9.6 (0.3–23.0)	–	–	Increased FA, probability, and displacement in young children with ASD	–
Keller et al. (2007)	34	31	18.9 ± 7.3	18.9 ± 6.2	102.0 ± 14.8	109.5 ± 9.0	Reduced FA in ASD in 5 regions of WM, including the corpus callosum, internal capsule, and forceps minor	FA positively correlated with age in ASD group in areas where group differences are found
Lee et al. (2007)	43	34	16.2 ± 6.7	16.4 ± 6.0	PIQ: 107.5 ± 13.0	PIQ: 112.8 ± 12.0	Reduced FA, increased MD, and increased RD in ASD in STG and temporal stem	Less age-related changes in FA of right STG in ASD group compared to TD group

Catani et al. (2008)	15	16	31 ± 9	35 ± 11	109 ± 17	120 ± 21	Reduced FA in cerebellum of Asperger's but no MD group differences	FA of left superior cerebellar peduncle negatively correlated with ADI-R social domain scores
Conturo et al. (2008)	17	17	26.46 ± 2.73	26.08 ± 2.69	104.41 ± 2.08	105.24 ± 2.34	Reduced RD in ASD in the right hippocampus-FG pathway. Increased RD and AD in ASD group in the left hippocampus-FG pathway and bilateral amygdala-FG pathways	Decreased across fiber diffusivity related to poorer Benton face interpretation and PIQ scores
Sundaram et al. (2008)	50	16	57.5 ± 29.2 months	82.1 ± 42.4 months	–	–	Increased apparent diffusion coefficient (ADC) in ASD in entire frontal lobe and in long- and short-range association fibers. Reduced FA in ASD group for short-range but not long-range fibers	–
Thakkar et al. (2008)	12	12	30 ± 11	27 ± 8	VIQ: 124 ± 12 PIQ: 120 ± 10	Estimated VIQ 114 ± 9	Reduced FA in ASD in WM near ACC	Greater ADI repetitive behavior scores related with decreased FA in WM near the left subgenual rostral ACC and related with greater fMRI activation during correct trials in the right rostral ACC
Brito et al. (2009)	8	8	Median 9.53 ± 1.83	Median 9.57 ± 1.36	–	–	Reduced FA in ASD in aspects of the corpus callosum, corticospinal tract, internal capsule, and cerebellum. Increased MD in ASD in aspect of the corpus callosum. Increased FA in aspect of putamen	–

(continued)

Table 8.7 (continued)

Reference	ASD sample size (*N*)	TD sample size (*N*)	ASD age in years (mean ± SD)	Control age in years (mean ± SD)	ASD IQ (mean ± SD)	Control IQ (mean ± SD)	DTI findings	DTI correlates
Cheung et al. (2009)	13	14	9.3 ± 2.6	9.9 ± 2.5	99.5 ± 21.9	111.9 ± 19.7	Reduced FA in ASD in aspects of prefrontal lobes, ventral and middle temporal lobe, and cerebellum. Increased FA in ASD in superior longitudinal fasciculus and left occipital lobe	ADI-R social and communication scores negatively correlated with FA in fronto-striatal-temporal regions and posterior corpus callosum. ADI-R repetitive behavior scores negatively correlated with FA near basal ganglia, TPJ, splenium of corpus callosum, and cerebellum, but positively correlated with FA in left precentral gyrus
Ke et al. (2009)	12	10	8.75 ± 2.26	9.40 ± 2.07	100.60 ± 18.79	99.83 ± 17.93	Reduced FA in ASD in aspects of inferior and middle frontal gyrus and STG. Increased FA in ASD in aspects of frontal lobe, middle temporal gyrus, and sublobar extra nuclear area	FA of right frontal lobe (sub-gyral) positively correlated with CARS in children with ASD. No significant correlations with ADI-R
Knaus et al. (2009)	14	20	16.09 Range: 11–19	14.10 Range: 11–19	VIQ mean: 103.29 PIQ mean: 102.57	VIQ mean: 119.00 PIQ mean: 113.00	Atypical functional language laterality more common in ASD. No diagnostic group differences in FA, but those with more typical leftward lateralization had greater FA in the arcuate fasciculus across both ASD and control groups	–
Lee et al. (2009)	43	34	16.23 ± 6.70	16.44 ± 5.97	107.5 ± 13.0	112.8 ± 12.1	Replicated results of Lee et al. (2007) and Alexander et al. (2007) using a T-SPOON voxel-based method	–

Pardini et al. (2009)	10	10	19.7 ± 2.83	19.9 ± 2.64	49.20 ± 6.94	–	Reduced FA and WM volume in ASD in left OFC. Reduced FA in ASD in WM near the ACC, the inferior and medial frontal gyri bilaterally, and the right superior frontal gyrus	In ASD group, IQ scores correlated positively with the mean FA values of the left orbitofrontal cortex network
Pugliese et al. (2009)	24	42	23 ± 12	25 ± 10	104.7 ± 12.05	121.2 ± 16.1	No group differences in FA and MD. Higher number of streamlines in Asperger's in the cingulum and inferior longitudinal fasciculus. Lower number of streamlines in Asperger's in the right uncinate	–
Barnea-Goraly et al. (2010)	13	11	10.5 ± 2.0	9.6 ± 2.1	85.9 ± 17.4	119.9 ± 13.3	No group differences in DTI measures between ASD and siblings. Reduced FA and reduced AD in ASD group and sibling group compared to control group in multiple regions across the frontal, temporal, and parietal lobes	No significant correlations were found between the ADOS and ADI-R subscale scores and FA values
Bloemen et al. (2010)	13	13	39 ± 9.8	37 ± 9.6	110 ± 15.7	115 ± 14.4	Reduced FA and increased RD in adults with Asperger's syndrome over large areas of the brain. Reduced MD in brainstem in Asperger's group	–
Cheng et al. (2010)	25	25	13.71 ± 2.54	13.51 ± 2.20	101.60 ± 18.91	109.04 ± 9.45	Increased and decreased FA in ASD across multiple areas of the brain. Interaction effect of age by group, occurring mostly in the frontal lobe	–
Fletcher et al. (2010)	10	10	14.25 ± 1.92	13.36 ± 1.34	VIQ:103.7 ± 18.55	VIQ:102.7 ± 9.52	Increased MD and RD in left arcuate fasciculus in ASD. Lack of typical asymmetry between left and right AF in ASD	–

(continued)

Table 8.7 (continued)

Reference	ASD sample size (*N*)	TD sample size (*N*)	ASD age in years (mean ± SD)	Control age in years (mean ± SD)	ASD IQ (mean ± SD)	Control IQ (mean ± SD)	DTI findings	DTI correlates
Kumar et al. (2010)	32	16	5.0 range: 2.5–8.9	5.5 range: 2.5–8.6	–	≥85	Reduced FA in ASD and DI groups across multiple areas, but no difference between ASD and DI groups. Longer uncinate and arcuate fibers in right hemisphere in ASD, whereas TD and DI children had longer UF and AF fibers in left hemisphere	Fiber volume of the UF positively correlated with GARS stereotypic behavior, and fiber length and fiber density of the corpus callosum positively correlated with Vineland communication
Lange et al. (2010)	30	30	15.78 ± 5.6	15.79 ± 5.5	109.57 ± 16.7	115.13 ± 12.9	Reversed hemispheric asymmetry in diffusion tensor skewness of the STG in ASD. Able to classify ASD from TD using the skew of the STG and temporal stem	–
Noriuchi et al. (2010)	7	7	13.96 ± 2.68	13.36 ± 2.74	92.71 ± 6.68	116.43 ± 9.50	Reduced FA and AD in ASD in the left DLPFC, posterior STS/TPJ, right temporal pole, amygdala, SLF, and occipitofrontal fasciculus	FA of the left DLPFC was negatively correlated with total SRS scores in children with ASD
Sahyoun et al. 2010a	9	12	12.8 ± 1.5	13.3 ± 2.45	101.4 ± 12.48	106.1 ± 8.56	Reduced FA in ASD in multiple WM tracts connecting with the frontal lobe. Increased FA in ASD in bilateral UF and right SLF	Group differences in the correlations between FA of different brain areas and task performance
Sahyoun et al. (2010b)	9	12	12.8 ± 1.5	13.3 ± 2.45	101.4 ± 12.48	106.1 ± 8.6	Reduced FA in bilateral inferior frontal gyrus-fusiform gyrus and right inferior frontal gyrus-middle temporal gyrus tracts in ASD. No group differences in performance on a visuospatial task, but fMRI group differences in frontal and temporal activation	–

Shukla et al. (2010)	26	24	12.7 ± 0.6 (SEM)	13.0 ± 0.6 (SEM)	VIQ: 105.6 ± 3.6 (SEM) PIQ: 109.5 ± 3.3 (SEM)	VIQ: 108.2 ± 2.6 (SEM) PIQ: 110.3 ± 2.5 (SEM)	Reduced FA, increased MD, and increased RD in ASD for whole-brain WM and multiple regions across the brain	Age negatively correlated with MD of the posterior limb of the internal capsule in both groups and with MD of the splenium in ASD. No correlations between DTI and ADOS/ADI-R scores
Sivaswamy et al. (2010)	27	16	5.0 Range: 2.6–9.0	5.9 Range: 2.6–8.9	–	–	Increased MD in ASD in bilateral superior cerebellar peduncle, and increased FA in right middle cerebellar peduncle. Increased FA in ASD in left inferior cerebellar peduncle (but decreased FA in right)	–
Weinstein et al. (2011)	21	26	3.2 ± 1.1	3.4 ± 1.3 (pediatric patients with normal conventional MRIs)	–	–	Increased FA and decreased RD in ASD in genu and body of corpus callosum, left SLF, and bilateral cingulum	–
Groen et al. (2011)	17	25	14.4 ± 1.6	15.5 ± 1.8	98 ± 18	105 ± 9	Increased overall MD in ASD. No difference in gray or WM volume	–

(continued)

Table 8.7 (continued)

Reference	ASD sample size (*N*)	TD sample size (*N*)	ASD age in years (mean ± SD)	Control age in years (mean ± SD)	ASD IQ (mean ± SD)	Control IQ (mean ± SD)	DTI findings	DTI correlates
Ingalhalikar et al. (2011)	45	30	10.5 ± 2.5	10.3 ± 2.5	–	–	~84 % specificity in classifying ASD from controls, using MD of the left middle occipital gyrus, right STG, right superior occipital gyrus, right insula, right middle temporal, right internal capsule, right inferior temporal, and left caudate and using FA of the right inferior occipital WM, the left fornix, the left SLF, the right inferior occipital gyrus, the right external capsule, the left caudate nucleus, the left hippocampus, the left posterior corona radiata, and left cuneus, and the right internal capsule	–
Jeong et al. (2011)	32	14	58.80 ± 22.64 months	67.36 ± 23.81 months	–	–	More curvature, higher bending, reduced FA, and increased RD in ASD in the bilateral arcuate, the bilateral uncinate, and genu of the corpus callosum (especially near the TPJ)	Negative correlations between curvature and FA in both groups in all three WM ROIs. Positive correlations between curvature and RD in ASD in all three ROIs, but only in genu of the corpus callosum in the TD group
Jou et al. (2011a)	10	10	13.06 ± 3.85	13.94 ± 4.23	91.0 ± 24.79	105.0 ± 17.83	Reduced FA in the arcuate and inferior longitudinal fasciculi, SLF, corpus callosum/cingulum, and inferior fronto-occipital fasciculus	–

Langen et al. (2012)	21	22	25.57 ± 6.08	28.45 ± 6.39	107.45 ± 15.08	109.82 ± 13.71	Reduced FA in ASD in left putamen tracts. Increased MD in ASD in right accumbens tract. ASD poorer accuracy on no-go portion of go/no-go task and smaller total brain WM volume	No relation of FA and repetitive behaviors on ADI-R or ADOS. Possible relation between FA and go/no-go task performance, but not found within either group alone
Lo et al. (2011)	15	15	15.2 ± 1.0	15.0 ± 0.8	108.4 ± 7.3	110.6 ± 10.2	Reduced generalized FA in ASD in the three corpus callosum tracts under investigation. Leftward asymmetry pattern observed in controls but not in the ASD	–
Pardini et al. (2012)	22	–	Age at post-therapy scan: 21.9 ± 0.5	–	PIQ at therapy onset 48.9 ± 1.6	–	Increased FA of the uncinate in those with ASD who highly adhered to the therapy versus those who moderately adhered to the therapy	FA of the uncinate positively correlated with both the difference between pre- and post-therapy autism symptom severity and the length of time in therapy but negatively correlated with the age of therapy onset

(continued)

Table 8.7 (continued)

Reference	ASD sample size (*N*)	TD sample size (*N*)	ASD age in years (mean ± SD)	Control age in years (mean ± SD)	ASD IQ (mean ± SD)	Control IQ (mean ± SD)	DTI findings	DTI correlates
Poustka et al. (2012)	18	18	9.7 ± 2.1	9.7 ± 1.9	111.0 ± 14.4	112.8 ± 14.9	Reduced FA in ASD in right SLF and bilateral uncinate	FA of left SLF and left uncinate negatively correlated with ADI-R communication and interaction scores. FA of right SLF negatively correlated with ADOS communication. FA of left fornix negatively correlated with ADOS communication and interaction. FA of right fornix negatively correlated with ADI-R communication
Shukla et al. (2011b)	26	24	12.6 ± 3.06 (SEM)	13.0 ± 2.94 (SEM)	VIQ: 106 ± 3.6(SEM) PIQ: 109.1 ±3.3(SEM)	VIQ: 108.2 ± 2.6 (SEM) PIQ: 110.3 ± 2.5 (SEM)	Reduced FA in ASD in the short-distance tracts of the frontal lobes. Increased MD and RD in ASD in the short-distance tracts of the frontal, temporal, and parietal lobes	Positive correlation between age and FA and negative correlation between age and MD and RD for short-distance tracts in each lobe for the TD group. However, for the ASD group, correlations are only significant in the frontal lobe

Shukla et al. (2011a)	26	24	12.8±.06(SEM)	13.0±0.6(SEM)	VIQ: 104.3±3.4(SEM) PIQ: 108.8±3.3(SEM)	VIQ: 108.2±2.6(SEM) PIQ: 110.3±2.5(SEM)	Reduced FA and increased MD for ASD in the anterior and posterior limbs of the internal capsule, the corpus callosum, inferior and superior longitudinal fasciculi, inferior fronto-occipital fasciculus, corticospinal tract, cingulum, and anterior thalamic radiation	FA of whole-brain WM positively correlated with age in TD group but only marginally in ASD. MD and RD of whole-brain WM negatively correlated with age in TD, but not in ASD
Ameis et al. (2011)	19	16	12.4±3.1	12.3±3.6	98.5±20.4	100.7±14.5	Increased MD and RD in cortico-cortical and interhemispheric WM tracts in ASD, especially in children and within the frontal lobe	–
Beacher et al. (2012)	28	30	Males: 32±10 Females: 32±7	Males: 28±8 Females: 32±8	–	–	Sex-by-diagnosis interactions in the corpus callosum, anterior cingulum, and corona radiata, with increased FA in TD males compared to females, but no sex difference in Asperger's	–
Bode et al. (2011)	27	26	14.7±1.6	14.5±1.5	–	–	Increased FA and reduced RD in ASD in right inferior fronto-occipital fasciculus and right optic radiation	FA not correlated with age
Verhoeven et al. (2012)	19	21 (+13 SLI)	13.8±1.6	10.1±0.4	–	–	FA of SLF similar in ASD language impairment and controls	FA of SLF not correlated with language scores in ASD with language impairment

(continued)

Table 8.7 (continued)

Reference	ASD sample size (*N*)	TD sample size (*N*)	ASD age in years (mean ± SD)	Control age in years (mean ± SD)	ASD IQ (mean ± SD)	Control IQ (mean ± SD)	DTI findings	DTI correlates
Hong et al. (2011)	18	16	8.7 ± 2.18	9.8 ± 1.9	105.2 ± 21.1	106.1 ± 20.1	Increased apparent diffusion coefficient and decreased fiber number in ASD in anterior third of corpus callosum	No relations between DTI measures and CARS
Cheon et al. (2011)	17	17	11.0 ± 2.1	10.2 ± 2.0	112.1 ± 12.0	113.8 ± 11.0	Decreased FA and increased MD in right anterior thalamic radiation, corpus callosum, and left uncinate fasciculus in ASD	FA of right anterior thalamic radiation and right uncinate negatively correlated with SRS scores within ASD group
Jou et al. (2011b)	15	8	10.9 ± 3.8	11.5 ± 2.6	–	–	Decreased FA in ASD in multiple tracts with the largest group differences occurring in the forceps minor, fronto-occipital fasciculus, and superior longitudinal fasciculus	No relation between FA and SRS after correcting for multiple comparisons
Lai et al. (2012)	16	18	11.02 ± 3.72	11.17 ± 4.39	Low-functioning and language impaired	Typically developing	Arcuate fasciculus tractography from Heschl's gyrus seed to inferior frontal gyrus: mean FA in left dorsal tract was lower in the autism group; no differences in FA in left or right ventral or right dorsal tract; no differences in MD	FA in left arcuate fasciculus correlated with fMRI activation in left inferior frontal gyrus for both speech and song
Lewis et al. (2012)	12 ASD with TSC	42 TD	0.5–25 years non-idiopathic ASD with Tuberous Sclerosis Complex (TSC)	0.5–25 years 30 TSC without ASD 42 TD	–	–	Tractography of arcuate fasciculus: FA decreased and MD increased in AF of non-ASD TSC compared to TD; FA more decreased and MD more increased in TSC with ASD	Greater differences in arcuate fasciculus in TSC + ASD than in non-ASD TSC

Nagae et al. (2012)	35	25	11.3 (6.7–17.5) years for $n=18$ ASD with no language impairment (LI); 9.6 (6.8–13.9) years for $n=18$ ASD with LI	11.4 (6.5–18) years	All scored >75 on the Perceptual Reasoning Index of the WISC-IV	Typically developing	FA: no significant group differences in superior longitudinal fasciculus (SLF), its temporal lobe part (tSLF), or corticospinal tract (CST) MD increased in Lt. SLF, particularly in Lt. tSLF	Increased MD in Lt. SLF and tSLF in ASD compared to TD was mainly due to increased MD in the ASD subgroup with impaired language
Wan et al. (2012)	5	5	6.7 ± 1.2	7.0 ± 0.9	All completely nonverbal	Typically developing	Tractography of arcuate fasciculus (AF) for tract volume: all typically developing children had significant Lt > Rt AF; 4 of 5 nonverbal autism children had Rt > Lt AF	Arcuate fasciculus volume shows an overall hemispheric reversal in completely nonverbal children with autism
Wolff et al. (2012)	92 infants high risk for ASD, 28 who met criteria at 2 years	64 infants high risk for ASD but did not meet criteria at 2 years	$N=28$ ASD at 24 months Time 1 age: 6.8 ± 0.8 months	$N=64$ No ASD at 23 months Time 1 age: 6.7 ± 0.8 months	Mullen at 24 months of age: 90.4 ± 23.7	Mullen at 24 months of age: 102.1 ± 15.7	Longitudinal prospective study with 3 time points: 6, 12, and 24 months. At 6 months of age, infants who developed ASD had increased FA in body of CC, left fornix, left uncinate, left inferior longitudinal fasciculus, and right posterior limb internal capsule. Tract growth rate was decreased in infants who developed ASD in 11 of the 15 tracts examined	

ACC anterior cingulate cortex, *AD* axial diffusivity, *ADC* apparent diffusivity coefficient, *ADI-R* autism diagnostic interview–revised, *ADOS* autism diagnostic observation scale, *ASD* autism spectrum disorder, *CARS* childhood autism rating scales, *CELF-3* clinical evaluation of language fundamentals-3rd edition, *CNTNAP2* contactin-associated like protein-2, *DLPFC* dorsolateral prefrontal cortex, *DI* developmental impairment, *DTI* diffusion tensor imaging, *FA* fractional anisotropy, *fMRI* functional magnetic resonance imaging, *GARS* Gilliam autism rating scales, *MD* mean diffusivity, *OFC* orbitofrontal cortex, *PIQ* performance IQ, *PDD-NOS* pervasive developmental disability-not otherwise specified, *RD* radial diffusivity, *ROI* region of interest, *STS/STG* superior temporal sulcus/gyrus, *TD* typically developing, *TPJ* temporoparietal junction

callosum, the frontal lobe, the temporal lobe, the midline structures, and the thalamic radiations. Similarly, there was some (but less substantial) evidence for group differences in areas such as the arcuate fasciculus, uncinate fasciculus, and cerebellar WM. These regions may be particularly prone to individual differences in both the ASD and typically developing groups. In addition, there is a preliminary evidence to date that suggests atypical lateralization in ASD and a different developmental trajectory for WM development in ASD. Future longitudinal studies are needed to explore how WM develops in ASD more thoroughly.

8.5.1 Limitations of the DTI Studies

Some caution should be used when interpreting what WM group differences indicate in terms of the biological basis of ASD. It is important to recognize that DTI measures are highly sensitive to a number of nonbiological factors, including pulse sequence parameters, field strength, signal to noise, spatial resolution, and diffusion weighting. These factors may make it particularly challenging to compare measurement values across sites and scan protocols. In addition, biological mechanisms including atypical myelination, axonal density, or axonal size may influence DTI measures though the specificity of the mechanism is very poor. More specific imaging measures of myelination, including magnetization transfer and the myelin water fraction from T2 relaxometry may help disambiguate specific mechanisms [see review in Alexander et al. (2011)].

8.5.2 What Do DTI Results Suggest About Neurodevelopmental Mechanisms in Autism?

The studies reviewed provide strong convergent evidence that WM microstructural changes are part of the developmental neuropathology of autism. Exactly what is going on at the molecular and cellular level is not known, but gene network studies, imaging-genetic studies, combined animal and human studies, newly developed methods to "dissect" and analyze individual fibers and better measures of myelin in postmortem studies, and pluripotent stem cell research are poised to help answer this critical question. There is also a strong evidence that early abnormalities of gray matter development are part of the primary pathology of autism (Casanova et al. 2002). Indeed, the cell bodies of all fibers in WM lie in gray matter. It is important to move beyond studying WM and gray matter separately in children and adults with autism and develop image analysis and multivariate statistical methods to understand the interaction between them.

Although the findings of increased FA in very young children with ASD needs validation by replication in additional independent samples, the findings to date suggest a wave of dynamic changes in WM microstructure during postnatal brain

development in autism. Longitudinal DTI results in older individuals will determine if the WM microstructural pathology of autism occurs early and then remains static across later development, or whether dynamic pathologic or compensatory changes continue into adulthood. It is still clear that there is much to be learned regarding WM integrity in ASD. Therefore, we highlight a couple of promising future directions.

8.5.3 Avenues for Future Research

8.5.3.1 Linking Structural and Functional Connectivity in ASD

A substantial amount of research has found evidence for decreased functional connectivity [for a review, see Schipul et al. (2011)] and decreased WM structural connectivity (Travers et al. 2012). However, very few studies have linked functional connectivity and DTI measures in persons with typical development or in persons with ASD. In persons with typical development, a handful of studies suggest a robust overlap in resting state functional connectivity and DTI measures (Greicius et al. 2009; van den Heuvel et al. 2009; Supekar et al. 2010; Gordon et al. 2011). However, to our knowledge, except for the preliminary results reported by Lai et al. (2012), relations between functional connectivity and DTI measures in ASD have not yet been reported, making this an extremely important avenue for future research.

8.5.3.2 Longitudinal Study of WM in ASD

Throughout the review, we mention the critical need for longitudinal studies to examine developmental trajectories of WM microstructure in ASD. Such a longitudinal design would be instrumental in determining the age-related biomarkers of ASD. Cross-sectional versus longitudinal studies of brain development can render quite different results (Brain Development Cooperative Group 2012), as longitudinal analysis of individual change over time is able to quantify within-person and between-person variation simultaneously and is able to greatly reduce previously unexplained variability. Such analytic advances enable us to better characterize similarities and differences in developmental trajectories of ASD compared to typical development. Longitudinal investigations also can determine continuities and discontinuities of changes in WM microstructure across development. They allow testing of potential mediating mechanisms and risk factors and can help elucidated causal pathways.

8.5.3.3 Treatment Intervention

Promising intervention results outside of ASD research show that WM integrity can change as a function of motor learning and practice (Taubert et al. 2010; Bosnell

et al. 2011), memory training (Engvig et al. 2011), reading intervention (Keller and Just 2009), or working memory, episodic memory, and perceptual speed training (Lovden et al. 2010). However, WM integrity changes as a function of ASD-specific interventions will have to be confirmed or denied by future longitudinal randomized controlled clinical trials. Pardini et al. (2012) offers very preliminary evidence suggesting that interventions may be able to affect WM integrity changes in ASD. In this study, 22 individuals with ASD completed an intensive communication intervention for many years, and WM integrity of the uncinate fasciculus was found to be related to the age when treatment began, treatment duration, and decreases in ASD symptom severity over treatment course.

8.5.3.4 Biological Classification and Prediction Research

Finally, a handful of studies have used DTI measures to distinguish between individuals with ASD and individuals with typical development. These studies have used the shape of corpus callosal splenium fibers (Adluru et al. 2009), tensor skew of the bilateral STG and right temporal stem (Lange et al. 2010), and tensor coefficients from many areas across the brain (Ingalhalikar et al. 2011) to successfully discriminate between ASD and typical development. These preliminary classification efforts offer hope that DTI methods may someday aid in the diagnosis of ASD. However, there is much research to be done in this domain. Although discriminating individuals with ASD from typically developing individuals is the first necessary step, clinically more important contributions of DTI will be helping to define and detect neurobiological subtypes of the disorder, distinguishing ASD and subtypes of ASD from other developmental and neuropsychiatric conditions, assisting in the discovery of genes and other factors involved, providing information about pathological mechanisms within individuals, and contributing to the development of "personalized medicine" for children and adults with the disorder, as well as early detection and prevention.

8.5.4 Conclusions and Implications

This extended review suggests that a number of studies have found decreased FA (a measure of fiber coherence) and increased MD (a measure inversely related to tissue density) in persons with ASD across a number of WM tracts. These findings have been relatively consistent in the WM of the frontal lobe, temporal lobe, midline WM structures, and thalamic radiations, and slightly less consistent in regions such as the uncinate fasciculus and cerebellum. Therefore, there is mounting evidence by these DTI and other studies that suggests decreased brain connectivity in ASD. The technologies for diffusion imaging of the brain and image analyses are rapidly advancing which will greatly improve the quality of WM characterization into the future. In addition, our hope is that DTI methodologies will expand to

include longitudinal designs, larger samples, and individual growth trajectories/behavioral mediators. As the number of DTI and other neuroimaging studies in ASD grow, the understanding of how brain connectivity and WM are affected in people with ASD will be improved.

The results also demonstrate the large amount of scientifically rich biological information included in DTI data. The summary measures described in the review likely represent only the "tip of the iceberg" of the biological information that will be discerned about ASD from advances in DTI in the near future.

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Biography

Brittany G. Travers is currently a T32 postdoctoral fellow at the Waisman Center, University of Wisconsin-Madison. She received her Ph.D. in cognitive psychology from the University of Alabama. Her research interests center around better understanding how WM atypicalities in autism spectrum disorder relate to the diversity of cognitive and behavioral phenotypes seen across the autism spectrum.

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Chapter 9
Spectroscopic Brain Imaging in Autism*

Janet E. Lainhart, Jason Cooperrider, and June S. Taylor

9.1 Introduction

In this chapter, we first provide simplified explanatory overviews of the spectroscopic techniques for nontechnical readers new to the field. In Sect. 9.1, we review published autism research studies that have used near-infrared spectroscopy (NIRS). This technique has only recently been applied to study autism spectrum disorders (ASDs). Section 9.2 is a detailed review of the more extensive literature about magnetic resonance spectroscopy (MRS) and MR spectroscopic imaging (MRSI) research in autism. We begin with a review of MRS/MRSI in young children with ASD (Sect. 9.3.1), because young children are closest in age to the primary initiating pathology of autism, which is still not known. Then, we discuss results

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from particular brain regions of high interest in autism across development, from childhood to adulthood (Sects. 9.3.2–9.3.7). In particular, the results of MRS/MRSI studies of the frontal lobes, cingulate, amygdala–hippocampal region, thalamus, and cerebellum are presented. In Sects. 9.3.8–9.3.13, we review specific demographic and other factors that could potentially influence and, at times, confound case–control differences in MRS/MRSI studies. We discuss what MRS/MRSI findings to date suggest about biological mechanisms involved in autism in Sect. 9.3.14. A table listing all MRS/MRSI studies of ASD used in the review and a glossary (Table 9.14) explaining common terminology and abbreviations frequently used in published MRS/MRSI literature follow the Conclusion.

9.1.1 Spectroscopy

This area of science, which is the basis for all of the imaging techniques described in this and the other imaging chapters, focuses on the interaction between a type of energy—electromagnetic radiation—and matter. Atoms and molecules emit and absorb electromagnetic radiation (EM radiation). Chemical nuclei such as hydrogen, carbon, and phosphorus, because of their unique nuclear and atomic structure, and their vibrational and rotational motion, have a ladder of specific energy levels. At equilibrium, they will preferentially settle in the lowest rungs of the ladder—their "ground states." They will absorb specific frequencies of radiation that correspond to the distance between rungs of their energy ladder; this absorption will promote them to an "excited state." The energy differences between rungs of the ladder are sets of frequencies specific to each nucleus. The frequencies of the energy they "best" absorb, and the time course of their excitation and relaxation back to baseline state when the energy source is removed, identify the nucleus and even the molecule in which the nucleus resides. The pattern of frequencies absorbed and/or re-emitted identifies particular molecules of interest in living tissues, such as water, oxygenated and deoxygenated hemoglobin, creatine, and glutamate. The electromagnetic radiation relevant to NIRS is in the "near-infrared" range of the electromagnetic spectrum. The near-infrared range is just below the energy range of visible light. For magnetic resonance, the resonant frequencies of nuclei are still lower on the electromagnetic spectrum, between FM radio and radar.

9.1.2 Near-Infrared Spectroscopy

When near-infrared (NIR) energy is absorbed by human tissue and transmitted back to a monitoring device, it captures and provides information about changes in blood hemoglobin concentration in the tissue and an important oxygen sensor, cytochrome c oxidase. While NIRS is noninvasive and does not require the long times that MRS does, it has its own set of limitations. The primary limitation is its shallow penetration into the brain: NIRS can only sample a depth of approximately 3–4 cm below the detectors, which are placed on the skull. This is purely a function of the

absorption and dispersion of the IR radiation within living tissue (Hoshi 2003; Ferrari et al. 2004). NIRS has perhaps even more difficult problems in quantitating its measures (of HbO_2 and cytochrome oxidase reduction) than MRS. Defining an effective attenuation and tissue path length is a major problem, due to variable attenuation from the skin/subcutaneous fat/bone layer covering the brain, and the effect of blood volume changes on the tissue path length and consequently on the observed sample volume. There are also difficulties in separating how much of an observed NIRS signal change is due to brain vs. scalp blood flow or to simultaneous changes in flow and volume sampled (Ferrari et al. 2004).

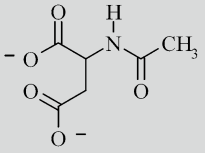
Although still being developed and tested, NIRS already has some clinical applications. It is also now being used in autism research, especially to study the neurobiology of the cortex in infants at high risk of developing autism and in young children with the disorder. Although its use in ASD research has been limited to the measurement of the hemodynamic response of the surface of the brain to neuronal activation (i.e., brain functioning), a review of the NIRS literature is included in this chapter because it is an application of spectroscopy.

9.1.3 Magnetic Resonance Spectroscopy and Spectroscopic Imaging

Magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI) measure the concentrations of specific biochemicals in the brain. Chemicals that can be measured include markers of neuronal integrity and homeostasis, cell membrane and myelin turnover, cell energy metabolism (including energy-producing compounds), osmotic regulation, acid–base balance, and some neurotransmitters. In the 1980s, brain metabolites were measured in large single volumes of interest that often contained heterogeneous structures and CSF as well as brain tissue. Technological advances in the past 10 years (Alger 2010)—MRI scanners with stronger magnetic fields (3 T, 7 T), phased-array head coils with much improved sensitivity, and parallel imaging techniques—allowed investigators to map the concentration of multiple metabolites across whole slices or slabs of the brain with greater speed and confidence. This makes it possible to analyze specific brain structures and tissue subtypes. There have been advances in MRI system quality assurance and in the quality control of MR spectral data (van der Graaf et al. 2008). Improved standardization and **absolute quantification** of metabolite concentrations by using brain water as a reference, rather than just being able to measure relative chemical concentrations expressed in ratios, make valid comparisons between studies possible. Advances include editing methods to separate the spectra of biochemicals that previously overlapped, enabling measurement of several specific neurotransmitters, rather than only their combined concentration. Among very recent advances are new protocols to measure biochemical concentrations in small brain structures, like the amygdala, much more reliably (Nacewicz et al. 2012). Dager et al. (2008) present an excellent review of MRS methodological considerations, approaches, and advances for research studies of psychiatric disorders.

NAA

N-acetylaspartate (NAA), a sensitive marker of the integrity of neurons and their homeostatic interactions with neuroglia, has been studied from early childhood to adulthood in autism. NAA is the most abundant neurochemical after glutamate and is manufactured in the mitochondria of neurons. Its main breakdown pathway is via transfer from neurons to oligodendroglia, which forms the myelin sheath, where it is broken down by the specific enzyme aspartoacylase (EC 3.5.1.15). Of all the neurochemicals of the human brain, NAA provides the greatest signal in MRS, because of its relatively high concentration in brain tissue and because the three identical protons of its methyl group yield a sharp single peak in the proton MR spectrum. Changes in NAA appear related to the density and metabolic health of neurons and their axons, including myelination. In typical development, levels of NAA increase dramatically in the brain during the first 2 years of life and then plateau, reach adult levels in the late teenage years, and decline during adulthood. Decreases in NAA are seen in disease states that involve injuring neurons and axons and traumatic injury of the brain. Increased NAA is thought to indicate increased neuron cell packing density or metabolism. When a change in NAA has been found in autism, it has almost always been a decrease.

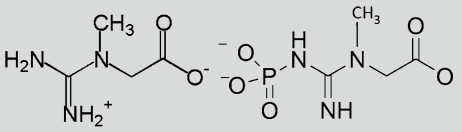


PCr and Cr

Phosphocreatine (PCr) is a naturally occurring biochemical in vertebrates and is used as an energy reservoir in cells, particularly cells in excitable tissues (muscle, brain, heart) that experience wide swings in energy demand. PCr buffers these swings, replenishing the primary cellular energy compound, adenosine triphosphate (ATP), by transferring its high-energy phosphate group to ADP. The MRS peak labeled creatine (Cr) is properly called total creatine (PCr + Cr). Creatine, the precursor to PCr, is primarily produced in the liver and kidney. MRS studies of autism have examined the spectral peak of total creatine concentration, which includes both creatine and phosphocreatine.

(continued)

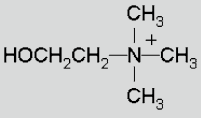
In typical development, these biochemicals increase during the first 2 years of life, remain at high levels during childhood, and decrease to adult levels in adolescence. Like NAA, these biochemicals usually decrease in brain regions affected by disease or trauma. Studies of Cr in autism present mixed findings that suggest it may be affected by developmental and IQ (or other disorder severity) effects.



Left, creatine; right, phosphocreatine

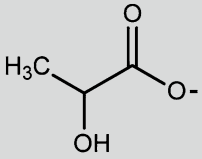
Cho

Choline (Cho) is a cation that must come largely from dietary sources. It is fount in compounds required for synthesis of membrane phospholipids (phosphorylcholine, PC) and also compounds resulting from membrane breakdown (glycerophosphorylcholine, GPC). Choline is also required for the synthesis of the neurotransmitter acetylcholine. The peak labeled Cho in 1H MRS actually includes negligible amounts of choline and is primarily PC + GPC, with 10–20 % contribution from phosphorylethanolamine (PE) and glycerophosphorylethanolamine (GPE), whose spectral lines are also at the CHO peak position. The ethanolamine compounds PE and GPE have similar metabolic roles to PC and GPC in membrane lipid metabolism. In keeping with common practice, we will refer to the sum of PC + GPC + PE + GPE as the Cho peak. These membrane precursors and breakdown products are found in highest concentration in myelin and neuroglia. They are somewhat elevated in inflammation. In typical development, the net concentration of Cho peak compounds in the brain increases during the first year of life as myelination increases, is high during childhood and decreases to adult levels by the latter part of adolescence. The concentration of Cho compounds in the brain increases when neurons are injured and there is increased turnover of cell membranes and myelin. In autism, MRS findings of Cho in the brain are mixed, with very young children with ASD showing different results from older children with autism and adults with Asperger disorder.



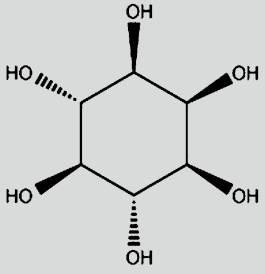
Lac⁻

Lactate (Lac^-) is an anion that forms when glucose (blood sugar) is broken down in your cells for energy during metabolic activity and exercise. Glucose is converted to pyruvate, which will eventually be broken down to provide additional energy if there is sufficient oxygen supply. However, when there is high energy demand—in hardworking muscles or brain regions—then pyruvate is converted to lactate, because the mitochondria cannot keep up with the demand to reduce oxygen to water. Along with glucose (which is transformed into lactate by glial cells in the brain), Lac^- is an important energy source for neurons in many species of mammals, including humans. Brain Lac^- can increase when metabolism in the brain is increased rapidly (e.g., during seizures) or disordered (e.g., from inborn errors of metabolism) or when the blood and oxygen supply to the brain is decreased (e.g., hyperventilation, hypoxia, vascular compromise). Studies of autism involving Lac^- have primarily been associated with mitochondrial dysfunction, and MRS studies of autism involving Lac^- have yielded no real differences in Lac^- levels between ASD groups and comparison groups.



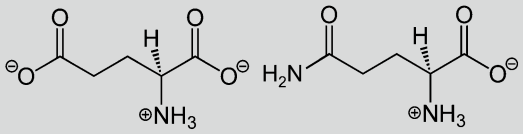
mI

Myoinositol (mI) is involved in maintaining the correct balance of water inside and outside of cells and in the transmission of chemical messages across cell membranes in the brain. It is produced in the human body from glucose, particularly in the kidneys, and can also come from dietary sources. It is thought to be produced when myelin degenerates and is also thought to be a potential marker of glial cells. Findings for mI from MRS studies of autism have been mixed.



Glx (Glutamate + Glutamine)

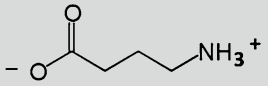
The 1H-MR spectra of the amino acids glutamate and glutamine show split peaks, called multiplets, because the protons in these molecules spin-couple to one another. As a result, at the magnetic fields currently used for human MRS (1–3 T), 1H MRS cannot distinguish between glutamine and glutamate. The peak for the combination of glutamate and glutamine (Glx) is small and overlaps the peak for GABA and the tail of the much larger NAA peak, so it is a challenge to determine accurately the area under the Glx peaks, which gives the Glx concentration. Glutamate is synthesized in the brain from glutamine in glial cells, travels into neurons, and functions as the primary excitatory neurotransmitter of the mammalian central nervous system. It is a key component of cellular metabolism and is a precursor of the inhibitory neurotransmitter GABA. Glutamate has been an important aspect of autism research, because of excitation-inhibition differences that might contribute to the neurobiology of autism. Glx has been measured in a number of MRS studies of autism, with mixed results.



Left, glutamate; right, glutamine.

GABA

Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter of the mammalian central nervous system and is synthesized from glutamate in the brain. Like glutamate, GABA has been an important aspect of autism research, because of excitation-inhibition differences that might contribute to the neurobiology of autism. In MRS studies of autism, GABA has been found to be reduced in various brain regions.



Expert Commentary

Stephen R. Dager, M.D.

Q: What has been the most important innovation in autism MRS/MRSI during the last 10 years?
A: The most important innovation in the use of MRS to study autism over the last 10–15 years would probably be the development and application of rapid chemical imaging techniques (MRSI) that allow for systematic assessment of regional and gray/white brain chemical concentrations. For example, our current work uses a 3-D chemical imaging technique (3-D proton echo-planar spectroscopic imaging) to concurrently acquire approximately 8,000 voxels co-registered to the brain anatomy and having excellent spatial resolution (0.3 cm^3 voxel size). The time frame required for our 3-D chemical imaging acquisitions, less than 5 minutes, is the same as generally used for single-voxel techniques. Coupled with this technological advance is the increasing recognition among researchers that autism is a developmental disorder necessitating that narrow age ranges be studied using MRS/MRSI (Posse et al. 2012, in press).

Q: What is the most exciting prospect for the next 10 years?
A: The most exciting prospect for MRS/MRSI studies of autism is the application of spectral editing techniques that are designed to isolate and measure specific chemicals, for example, GABA, glutamate, and glutamine that otherwise cannot be separated due to overlapping spectral peaks. These newer editing approaches are allowing us, for example, to acquire rapid 2-D and 3-D GABA images in clinically acceptable time frames from sleeping infants (Posse et al. 2012, in press; Prescot et al. 2012).

Q: What are the major challenges that must be overcome to achieve this?
A: I would say that the major challenges that must be overcome to maximize the scientific insights gained from MRS/MRSI are the use of more consistent methodology across studies, obtaining larger sample sizes through multi-site studies, controlling for potentially confounding variables, and employing longitudinal designs capable of tracking changes in neurochemical concentrations over the course of development and across the life span.

9.2 Near-Infrared Spectroscopy in ASD

Table 9.1 summarizes the 5 NIRS studies of ASD published to date.

Table 9.1 NIRS studies of ASD

References	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Cortical regions of interest	Stimuli	Results
Minagawa-Kawai et al. (2009)	9 7M, 2F	9 (7 males, 2 females)	9.2 ± 1.8 6–11	Autism (DSM-IV, autism screening questionnaire) (all right handed; 4 had IQ<70)	TD controls (all right handed)	Bilat temporal auditory areas	Three different forms of Japanese verb for "go." Affirmative: "itta" (she has gone); imperative: "itte" (go away); interrogative: "itta?" (has he/she gone?) Phonemic contrast: itta vs. itte Prosodic contrast: itta vs. itta?	Cerebral laterality for phonemic processing: significantly weaker leftward lateralization in autism Cerebral laterality for prosodic processing: no group difference (both had less leftward, greater rightward lateralization) Conclusion: less leftward lateralization of phonemic processing in autism might impair language development and be due to abnormal cortical specialization for phonemic processing; left cortex hypoactivity or right cortex hyperactivity

(continued)

Table 9.1 (continued)

References	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Cortical regions of interest	Stimuli	Results
Kawakubo et al. (2009)	27	24 and 27	16.2 ± 6.7 5–39	High-functioning ASD (DSM-IV; CARS; IQ ≥ 85)	24 healthy non-affected siblings of ASD participants 27 TD controls	Prefrontal cortex (approximately BA10; frontopolar regions/ anterior prefrontal cortex)	Executive processing letter fluency task: generate as many words beginning with a syllable such as "a" as they could in 30 s	Child group: no significant differences in change in oxy- or deoxyhemoglobin (micromol) Adult group: average change in oxyhemoglobin was less in the ASD adults than in TD controls; non-affected siblings had intermediate values Conclusion: given the limitation of cross-sectional design, the results suggest altered age-related prefrontal activity during executive processing in ASD, and sibling data suggest some genetic factors might influence the developmental PFC deficit

Kita et al. (2011)	10 males	13 boys 11 men	10.2 ± 1.1 9–12	ASD (high-functioning autism or Asperger) (DSM-IV)	Healthy male children and adults	Probes and channels on forehead to detect inferior frontal cortical activity	Morphing movies of self-face to unfamiliar face and familiar face to unfamiliar face: passive NIRS monitoring of self-face recognition while focusing on sex changes of face	ASD children performed self-face recognition as well as control children and had similar frontal hemodynamic activities. Children with more severe ASD had less hemodynamic activity than children with less severe ASD
Suda et al. (2011)	–	28 14M, 14F	–	–	All TD age 26.4 ± 3.0 23–35 years	Prefrontal cortex and superior temporal sulcus	Face-to-face conversation, semi-structured, about food (realistic social interaction)	In the TD sample: prefrontal cortex and STS were significantly activated during face-to-face conversation, especially in males TD participants with higher levels of autistic traits on the Autism Spectrum Quotient had less brain activation in the STS during face-to-face conversation

(continued)

Table 9.1 (continued)

References	ASD sample size (*N*)	Control sample size (*N*)	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Cortical regions of interest	Stimuli	Results
Funabiki et al. (2012)	11 10M, 1F	12 10M, 2F	16.8 ± 6.1	ASD (Asperger or high-functioning PDD) (DSM-IV; no language delay or cognitive subnormality—MR)		Prefrontal and temporal cortices	Tones and then voices during meaningful stories; session 1—instructed to listen attentively; session 2—instructed to ignore and not listen	ASD participants had typical auditory cortex activation response to sounds and voices when attending to them Conclusion: apparent lack of awareness of voices/sounds in ASD might be due to deficits in regulating attention in response to voices, rather than primary deficits in the auditory cortex

9.3 MRS and MRSI in ASD

Where Does the MR signal Come from?

Magnetic resonance imaging (MRI) in its many clinical forms is based on detecting energy transitions (specifically, transitions between nuclear spin energy states) in the pair of hydrogen nuclei in mobile water molecules—wherever the water is—inside cells or outside, in CSF or blood. The majority of magnetic resonance spectroscopy (MRS) studies of brain detect the hydrogen nuclei (protons) in metabolites as well as water—thus the name 1H MRS (proton MRS). It is possible to obtain MR images or MRS from nuclei other than protons, although this is much more difficult because their signals are so much weaker. MR imaging has been successfully obtained from fluorine-containing compounds (^{19}F MRI) and sodium ions (^{23}Na MRI), although these are presently used only in research. There is a much larger body of MRS studies with other nuclei—primarily phosphorus (^{31}P), fluorine (^{19}F), and carbon (^{13}C)—which have contributed very significantly to our understanding of animal and human metabolism.

What Is the Biggest Difference Between MRI and MRS?

The concentration of water protons in tissue is ~83 M. The high end of proton concentrations in the brain metabolites we can observe with 1H MRS is 24–30 mM for NAA methyl protons (Pouwels and Frahm 1997) and 15 mM for the nine equivalent protons of the cholines (Howe et al. 2003). These concentrations are .02–04 % of water proton concentrations. Because the detected MRS signal is proportional to concentration, that means the signals for 1H MRS, even from metabolites of high concentration, are ~10^{-4} weaker than those for MRI. Repeating the MRS measurement 25 times and doubling the strength of the magnetic field can produce only a factor of 10 increase in the MRS signal. The only practical way to make up the remaining three orders of magnitude is to make the voxel size for MRS about 1000 times larger than that of MRI. Thus if the **voxels** (spatial resolution) in an MR image are approximately 2×2×2 mm, an MRS voxel size to get the corresponding signal would be ~2×2×2 cm. In practice, the situation is even worse than this, because MRI data can be collected much more quickly than MRS. So MR spectra always have much more **noise** than MR images, and the voxel volumes, and therefore anatomical resolution, are many times worse than the sharply clear images from MRI.

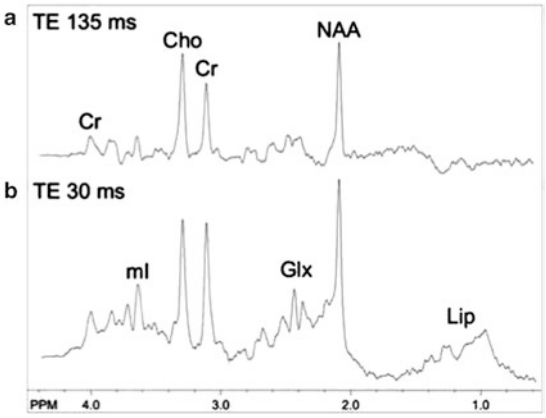


Fig. 9.1 The influence of echo time TE on 1H-MR spectra of human brain. (a) At long TE = 135 ms, the single large peaks for the methyl ($-CH_3$) groups of the metabolites NAA, Cr_T, and Cho_T are clearly visible. (b) At short TE = 30 ms, the smaller mulitplet peaks of mI and Glx are also visible (From (Stanley 2002), used with permission)

Do MRI and MRS Give the Same Kind of Information?

Not really. MRI produces beautiful, sharp images in which the anatomy and regions of the brain are easily distinguished—gray from white matter from CSF, the shape of the gyri and sulci of the cortex, the sweep of the fiber tracts of the striatum and corpus callosum, and abnormalities like tumors, abscesses, or malformations. MRI collected after injection of a contrast agent will also show defects in the blood–brain barrier. On the other hand, MRS produces squiggly lines with multiple peaks (Fig. 9.1) and tables of numbers—not nearly as sexy as MRI nor as easy to display as a function of brain anatomy. However, what MRS provides that MRI cannot is a biochemical analysis of the living brain. Instead of producing a single intensity value for each voxel, MRS yields a spectrum of multiple peaks for each voxel. The positions of the peaks in a spectrum give us the identity of particular compounds. The positions on the X-axis are labeled in frequency units unique to MRS: parts per million, or PPM. PPM is the frequency of the resonance line (in Hz) divided by the main resonance frequency of the particular magnet in which the MRS was measured (in megahertz, MHz). The PPM scale makes all the peak positions identical, no matter what the magnetic field strength is. In Fig. 9.1, the peaks in 1H MRS spectra from human brain are labeled to identify those from brain metabolites. Single peaks—e.g., from methyl ($-CH_3$) protons—show up at both short and long echo times (**TE**), whereas the more complex multiplet lines from the coupled protons of Glx and mI can only be seen in the short-TE spectrum. The areas of those peaks are proportional to the concentration of the compounds, *ceteris paribus*. MRS results can be expressed in numerical form: as tables of calculated metabolic concentrations (determined by relative or "absolute" quantification).

How Does MRS Determine What Metabolites Are Present and Their Concentrations?

Identifying what biochemical metabolites are present from an MR spectrum is easy—each metabolite has a characteristic pattern of peaks in the MRS spectrum that identify it. In Fig. 9.1, the peaks in 1H MRS spectra from human brain are labeled to identify those from brain metabolites. Calculating how much of each metabolite is present is much more difficult.

Concentration C of metabolite M in a volume of brain V_B is

$$C_M = (\text{moles of M}) / V_B$$

The key idea to remember is that the area under M's peak in the MR spectrum, A_M, is proportional to M's concentration, C_M:

$$C_M = kA_M / V_B$$

The peak area A_M can be determined by automatic curve-fitting programs such as **LC Model**. V_B, the volume of brain tissue in the voxel, can be estimated by **partial-volume analysis**. The real problem is determining k, which depends on some factors that are specific to the MRI scanner and pulse sequence used to acquire the MRS, and other factors specific to the metabolite in a particular tissue in a particular brain. The only way to estimate C_M is to use a ratio of the metabolite peak area to some reference signal (Alger 2010). If the estimates are presented as ratios of metabolite concentrations (e.g., NAA/Cr), then the reference signal is shown explicitly—here, it is the Cr peak area. Use of ratios with Cr as the denominator may cause errors in interpretation if Cr is affected by the disorder (Pfefferbaum et al. 2000; Dager et al. 2008) or if there are significant regional variations in Cr (Nacewicz et al. 2006). There is evidence that Cr is affected in autism (Friedman et al. 2003; Page et al. 2006). If a single metabolite concentration is presented, rather than a ratio of metabolites, it was still calculated from a signal ratio—but the reference signal might be the area of the water signal in the spectrum (internal reference) or the area of water or some other reference peak in a **phantom**. In addition, the concentration estimate or ratio of metabolites may or may not have been corrected for relaxation effects. Differences in reference signals and corrections for relaxation or partial-volume effects complicate comparisons between studies. In spite of these complications, a careful and quantitative analysis of MRS/MRSI should be used to obtain the best estimates of metabolite concentrations in studies of brain development, because differences between normal and abnormal brain chemistry may be subtle. The studies reviewed here have analyzed MRS data to produce tables of metabolite concentrations for ASD and control groups, with varying attention to relaxation and other factors that can affect the validity of the concentrations.

(continued)

Since the metabolite lines in 1H MRS are essentially anthills on the side of the Mount Everest of the water line, methods must be found for coping with this huge mismatch in size. There are several methods that suppress the waterline during acquisition of the MRS data, so that it is reduced in size to a percent or less of its normal value. It is also possible to remove a considerable amount of residual water signal by simple post-processing methods—that is, after the MRS data has been acquired. The water line's position in living tissue at body temperature is ~4.7 ppm, and you will note that virtually all published proton MRS spectra only show the spectral region of interest from 0.0 to 4.0 ppm. The wings of the water line extend out on either side of its peak and distort the baseline significantly to 4.0 ppm, especially for short-TE spectra. When attempting to measure concentrations of metabolites from lines that are close to the water resonance, one has to be careful that the method used to suppress the water line does not affect the area of those metabolite lines close to 4.0 ppm.

9.3.1 Very Young Children with ASD

Two research groups have examined brain chemistry in young children with ASDs. The first group (Friedman et al. 2003, 2006) used **MRSI** to map concentrations and T2 relaxation times of brain chemicals across many 1 cm^3 voxels in two contiguous 2 cm thick slabs of the brain in 45 children with ASD, compared to 13 typically developing (TD) and 15 non-autistic developmentally delayed (DD) children. The children studied were in the narrow age range 3–4 years of age. Methodologically, the study was well done, with excellent quantitation of metabolites, and the results are important. Comparison of the ASD group to the TD children identifies abnormalities in the ASD sample. Inclusion of the non-ASD DD children provides information about abnormalities that appear to be *specific* to ASD (i.e., found in the ASD group but not the DD group) and abnormalities that seem to be *shared* by the autism and DD samples, with both being abnormal compared to TD.

Metabolite concentrations were calculated relative to tissue water (Barker et al. 1993). Spectra in all 704 of the 1 cm^3 voxels in the imaging slabs of brain were averaged to give whole-brain (global) metabolite concentrations. When these global concentrations are compared between groups, Cr was specifically decreased in the ASD group relative to TD; Cr was not decreased in the DD group. Average NAA and mI were nonspecifically decreased in the ASD sample; quantities of these biochemicals were decreased in both the ASD and DD groups relative to TD (Friedman et al. 2003). Average Lac^- and combined glutamine–glutamate (Glx) concentrations did not differ between the three groups.

Biochemical concentrations were then examined in gray matter and white matter separately. The results are shown in detail in the middle row of Table 9.2. Most of

Table 9.2 Summary of specific and nonspecific abnormalities and absence of abnormality by level of analysis from global to regional in young children with ASD (Friedman et al. 2003, 2006)

Level of analysis	Abnormal and specific to ASD in young children	Abnormal but not specific to ASD in young children	Not found to be abnormal in young children with ASD (Corrigan et al. 2012) (first time point)
Whole MRSI brain slab volume (combined GM and WM) 20 mm thick, containing 7,04 1 cm^3 voxels	– Cr	– NAA – mI	Lac⁻Glx (glutamate+glutamine)
Total GM in slab	– NAA – Cr – mI + Cho T2	– Cho	Lac⁻
Total WM in slab		– NAA – mI	Lac⁻
CSF in slab			Lac⁻
Regional analysis (by brain structure)	– NAA bilateral cingulate – NAA thalamus + NAA T2 thalamus	– NAA left frontal white matter – NAA right parietal white matter	*NAA*: Right frontal WM, left parietal WM, and CC, insula, STG, medial temporal lobe, caudate, putamen, and midline occiput
	– Cr insula – Cr left parietal white matter – Cr thalamus – Cr anterior corpus callosum	– Cr left frontal white matter – Cr T2 posterior corpus callosum	*Cr*: Right frontal WM, right parietal WM, cingulate, right insula, STG, medial temporal lobe, caudate, putamen, and midline occiput
	– Cho right superior temporal gyrus – Cho right medial temporal lobe + Cho T2 right medial temporal lobe		*Cho*: Frontal WM, parietal WM, and CC, cingulate, insula, left STG, medial temporal lobe, caudate, putamen, and midline occiput
	– mI right insula – mI left parietal white matter – mI bilateral caudate – mI midline occiput – mI anterior corpus callosum		*mI*: Frontal WM, left parietal WM, posterior CC, cingulate, left insula, STG, medial temporal lobe, thalamus, putamen

45 ASD, all IQs (high and low functioning); age 3–4 years 10–13 typically developing, 12–15 developmentally delayed children; males and females, age 3–4 years (ASD and DD children received propofol for scanning)

the abnormalities in gray matter, but none of the abnormalities in white matter, were specific to ASD. NAA was specifically decreased in gray matter in the young ASD children; it was nonspecifically decreased in white matter. Cr and Cho were only decreased in gray matter. The decrease in Cr was specific to ASD. The decrease in Cho was nonspecific; it was found in children with ASD and in DD children, although the magnitude of decrease was significantly greater in the ASD group. Decreased mI was found in gray matter and white matter in the ASD children, but was specific to ASD only in gray matter. No differences in concentrations of Lac^- or Glx were found when ASD and DD children were compared to each other or when they were individually compared to TD controls. Lac^- was measured first by traditional methods (Friedman et al. 2003) and later by improved **spectral editing techniques** (Corrigan et al. 2010; 2012).

Finally, the group compared concentrations of the brain metabolites in specific brain regions. The results are shown in Table 9.3, with abnormalities specific to ASD bolded and abnormalities shared by ASD and DD in gray. The thalamus had the highest number of abnormalities specific to ASD, followed by the medial temporal lobe and anterior corpus callosum. The putamen was the only region examined that had no abnormalities in either the left or right hemispheres. The most frequent type of chemical abnormality specific to ASD was a decrease in mI, which was found in five regions. When absolute concentrations of the biochemical were abnormal, the concentrations were always decreased compared to the TD group. In contrast, abnormalities of T2 relaxation occurred in opposite directions; NAA and Cho T2 relaxations were prolonged in the thalamus and medial temporal lobe, respectively, and specific to the ASD group. Cr T2 relaxation was decreased in the posterior corpus callosum in both the ASD and DD groups. The investigators had hoped to examine the biochemistry of orbitofrontal, dorsolateral prefrontal, and medial frontal cortex, but spectral data in these areas were not sufficient.

Table 9.2 summarizes specific and nonspecific abnormalities at the whole-slab gray matter and white matter and regional levels. Most of the nonspecific abnormalities occurred in white matter.

Limitations of the above studies, mentioned by the investigators, include the small sample sizes of the TD and DD groups. Because "abnormality" in ASD is defined relative to the DD and TD groups, larger samples of DD and TD children are needed to make sure the TD and DD samples are representative of the populations from which they are drawn. The small sample sizes, in large part, are due to the difficulty involved in ascertaining children with "nonspecific" DD and scanning and collecting quality MRSI data from 3- to 4-year-old children in general. The research to date represents a major accomplishment. MRSI data in even younger children and infants at risk of developing autism (because they have an older sibling with autism) will likely be forthcoming from this research group.

Another team of investigators (Zeegers et al. 2007) examined an ASD sample with similar mean age (3.6 years) but slightly wider age range (2 to 6 years) and compared their study to the studies on the sample described above. They measured NAA, Cr, and Cho and their ratios in two single 1.5 cm^3 voxels placed in left frontal subcortical WM and the left amygdala–hippocampal region. In this more recent

Table 9.3 Regional *N*-acetylaspartate, creatine, choline, and myoinositol quantification and T2 relaxation in very young children with autism spectrum disorder compared to typical and developmentally delayed children (Friedman et al. 2003, 2006; Corrigan et al. 2012)

Groups Compared	Frontal GM OFC DLPFC MFC		Frontal WM	Cingulate	Insula	STG	Medial temporal	Parietal WM	Thalamus	Caudate	Putamen	Midline occiput	Corpus callosum
ASD vs. TD	excluded	NAA	Lt: - NAA	**Bilat: -NAA**		Rt. –NAA		Rt: -NAA	**Rt: -NAA Rt +NAA T_2**				
		Cr	Lt: - Cr		Lt: -Cr		Lt. +Cr T_2	Lt: -Cr	Lt: -Cr				Anterior: -Cr Posterior: - Cr T_2
		Cho				Rt: -Cho	Rt: - Cho + Cho T_2		Lt: - Cho				
		ml			**Rt: -ml**			**Lt: -ml**		**Bilat: -ml**		**-ml**	**Anterior: -ml**
DD vs.TD	excluded	NAA	Lt: - NAA					Rt: -NAA				- NAA T_2	Posterior: - NAA T_2
		Cr	Lt: - Cr										Posterior: - Cr T_2
		Cho								Rt. - Cho T_2			
		ml	Rt. -mL		Rt. –mL								
ASD vs. DD	excluded	NAA		Lt: + NAA T_2		Rt: + NAA T_2						-NAA + NAA T_2	
		Cr				Rt: + Cr T_2		Lt: + Cr T_2	Bilat: + Cr T_2				
		Cho			Lt. +Cho T_2	Rt. +Cho T_2	**Rt. + Cho T_2**		**Lt. + Cho T_2**		Lt. +Cho T_2		
		ml				Rt. -mL			Rt. -mL				

Lt left, *Rt* right, *Bilat* bilateral
– NAA, decreased NAA; + NAA T2, prolonged NAA T2 relaxation; –NAA T2, reduced NAA T2 relaxation
– Cr, decreased Cr (creatine and phosphocreatine); + Cr T2, prolonged Cr T2 relaxation; – Cr T2, reduced Cr T2 relaxation
– Cho, decreased Cho; + Cho T2, prolonged Cho T2 relaxation; – Cho T2, reduced Cho T2 relaxation
– mI, decreased myoinositol
All changes listed in this table are significant at the $p = 0.01$ level
Empty cell = no significant group differences
Bold = abnormality specific to ASD compared to TD
Gray = abnormality shared by ASD and DD compared to TD

study, an ASD sample ($n=25$) was compared to a DD sample ($n=12$; 4 with intellectual subnormality (i.e., MR) and 8 with a language disorder). No TD control group was included. The investigators found no case-comparison group differences and concluded their results did not replicate the findings of differences in NAA, Cr, and Cho in ASD in studies published before their report. Reexamination of the data, however, suggests that despite methodological differences between the Zeegers et al. (2007) and Friedman et al. (2003) studies, the results of the two studies were the same for the two regions examined in both studies: no differences were found in frontal lobe WM or the medial temporal lobe when the ASD groups were compared to the non-autistic DD children. Zeegers et al. found no difference in biochemical concentrations in the left frontal lobe WM between ASD and DD/LD controls. Friedman et al. found the same results. But, when Friedman et al. compared their ASD and DD samples to a TD group, it became evident NAA was abnormal in both ASD and DD groups. In the left medial temporal lobe, both studies found no difference between ASD and DD. Friedman et al. (2003) also showed that neither ASD nor DD differed from the TD group. Together, the results of the two studies suggest that one cannot reliably conclude brain chemical levels measured by MRS are normal or abnormal in autism when examination is limited to single MRS voxels, because abnormalities might be present in areas of the brain not examined. MRSI, which maps spectra across the brain, shows that biochemical abnormalities are present in some but not all brain regions in young children with autism. In addition, we cannot conclude abnormalities are absent when an ASD group is only compared to a non-autistic DD group; an abnormality could be present in both groups; a TD sample is essential. A design that includes non-autistic DD and TD comparison groups appears essential, but is very labor and resource intensive in participant recruitment and scanning and MRS/MRSI data analysis.

9.3.2 MRS/MRSI Findings in Gray Matter and White Matter Across Development in ASD

Only two studies have quantified biochemical concentrations in total gray matter and total white matter in large slabs of brain scanned with MRSI. The first was the Friedman et al. (2006) study of 3–4-year-old children with ASD. The second study by DeVito et al. (2007) examined children and adolescents 6–17 years of age (mean age 9.8 years). Despite differences in the samples in regard to sex, range of intellectual ability of the ASD samples, type of ASD, and sedation used for scanning, comparison of the cross-sectional findings shown in Table 9.4 indicates two possible important age-related trends (and, by cautious inference, developmental changes). Replication and testing in other samples is needed before anything other than preliminary observations can be made.

First, significantly decreased NAA was found in both studies, suggesting that, at the level of total gray matter, decreased NAA is present early in the course of autism and persists across development. This may imply decreased density of neurons and

Table 9.4 Gray matter and white matter brain metabolite quantification in young children and older children/adolescents with autism spectrum disorder compared to typical and developmentally delayed children[a]

		Young children with ASD (Friedman et al. 2006) 1.5 T MRSI		Older children and adolescents with autism (DeVito et al. 2007) 3.0 T MRSI	
		$n=45$ ASD, all IQs, males and females, mean age 3.9 years, range 3–4 years; 10 TD, 12 DD (ASD and DD children received propofol for scanning)		$n=26$ autism, pIQ ≥ 70, males, mean age 9.8 years, range 6–17 years; 29 TD, mean age 11.1 years (18 autism participants received midazolam for scanning)	
Groups compared		Gray matter	White matter	Gray matter	White matter
ASD vs. TD	NAA	**– NAA (–4.8 %)**	**– NAA (–5.1 %)**	**– NAA (–6.4 %)**	No case–control difference
	Cr	**– Cr (–6.5 %)**	No case–control difference	No case–control difference	No case–control difference
	Cho	**– Cho (–17.4 %) + Cho T2 (+12.4 %)**	No case–control difference	No case–control difference	No case–control difference
	mI	**– mI (–10.3 %)**	**– mI (–10.7 %)**	No case–control difference	No case–control difference
	Glx	No case–control difference	No case–control difference	**– Glx (–8.9 %)**	No case–control difference
	Lac^-	No case–control difference	No case–control difference	NA	NA
DD vs. TD	NAA	No case–control difference	**– NAA (–6.4 %)**	NA	NA
	Cr	No case–control difference	No case–control difference	NA	NA
	Cho	**– Cho (–11.5 %)**	No case–control difference	NA	NA
	mI	No case–control difference	**– mI (–12.3 %) trend**	NA	NA
ASD vs. DD	NAA	– NAA (–3.5 %) trend	No case–control difference	NA	NA
	Cr	No case–control difference	No case–control difference	NA	NA
	Cho	– Cho (–6.7 %)	No case–control difference	NA	NA
	mI	– mI (–10.6 %)	No case–control difference	NA	NA

[a]Bold type indicates significant difference between groups

their projections in GM. Second and even more important is the possibility that Glx in gray matter might not be abnormal early in ASD but becomes so over time. This possibility, which absolutely needs further study, is important because many children with autism remain impaired into adulthood for reasons that are not currently understood. The clinical course of some individuals with autism actually worsens over time, and seizures and neuropsychiatric complications emerge. At least one longitudinal MRSI study is in progress and, to date, only Lac^- findings are published from that study. No abnormalities in Lac^- concentration were found at, or developing across, any of the 3 time points studied: 3–4, 6–7, and 9–10 years (Corrigan et al. 2012). This study has already yielded one important finding: it is reasonable to conclude from these data, as the investigators did, that "no 1H MRS or MRI evidence for brain mitochondrial dysfunction, or shifts in brain oxidative metabolism, were observed in this sample of children with ASD at 3–4, 6–7 or 9–10 years-of-age. Our findings do not support the widespread and increasingly common use of hyperbaric oxygen to treat ASD, advocated on the basis of this presumed relationship."

9.3.3 MRS/MRSI Findings in the Frontal Lobe Across Development in ASD

The frontal lobe is the part of the brain most frequently examined in MRS/MRSI studies. Table 9.5 summarizes the studies and results by mean age of ASD participants. NAA is the biochemical most often examined and most frequently found to be abnormal. Six of 13 studies that quantified NAA concentration found ASD-control differences; in five of the six studies, absolute NAA concentration or NAA concentration relative to Cr concentration was decreased. In one study that included the oldest participants and only individuals with Asperger disorder, NAA was increased; however, in a similar study that included adolescents as well as adults with Asperger disorder (Öner et al. 2009), no difference in NAA was found. One-third (3 of 9) of studies measuring Cr found ASD-control differences; in two studies, one using ^{1}H MRSI (Murphy et al. 2002) and another using ^{31}P MRS (Minshew et al. 1993), total Cr and PCr were decreased, respectively; in the study of older individuals with Asperger disorder, Cr was increased. Only 10 % of the ten studies measuring Cho found it to be abnormal: Cho was increased in the older individuals with Asperger disorder. mI was abnormal in one of five studies and only relative to Cr. Table 9.6 summarizes frontal lobe NAA results by level of analysis, from more global to more specific regions.

Although only two studies have examined Glx and two studies have examined concentrations of GABA in the frontal lobe, all of these studies found Glx and GABA to be decreased. One of the Glx studies (DeVito et al. 2007) measured the absolute concentration of Glx in frontal lobe gray matter in children with ASD 6–17 years of age (mean age 9.8 years); the other study (Kubas et al. 2012) measured the Glx/Cr ratio in the left and right frontal lobes of children with ASD 8–15 years of

age (mean age 10.5 years). Harada et al. (2011) found the absolute concentration of left frontal lobe GABA was decreased compared to pediatric controls in ASD children 2–11 years of age (mean age 5.2 years). Kubas et al. (2012) found that the concentration of GABA relative to Cr was decreased (decreased Glx/Cr) in the left and right frontal lobes of the ASD children, 8–15 years of age. Because there is evidence that the balance of excitatory and inhibitory neurotransmission might be disturbed in at least some cases of ASD (Rubenstein 2010), MRSI studies of Glx, and specifically glutamate, as well as GABA, could provide critical information about frontal lobe development in ASD, with implications for mechanisms, neurobiological subtypes, and treatment (Ben-Ari et al. 2012; Hardan et al. 2012).

9.3.4 MRS/MRSI Findings in the Cingulate Across Development in ASD

The cingulate cortex has been of interest in autism because of its roles in autonomic control, emotion, response selection and assessment of the reward value of behaviors, attention, pain processing, and memory (Vogt 2005). Table 9.7 outlines the results of MRS/MRSI studies of the cingulate. Of the seven studies of NAA in the cingulate, only two have found concentrations to be abnormal (Friedman et al. 2003; Fujii et al. 2010). Both studies were in children and both found decreased NAA, either absolute concentration of NAA or, specifically in the anterior cingulate cortex (ACC), decreased NAA relative to Cr and to Cho. Five studies measured Cr in the cingulate and none of them found ASD-control differences. Two of six studies that quantified Cho found ASD-control differences, but in opposite directions. One of three studies measuring mI found it to be increased in the ACC. An important preliminary study of Glx in the ACC found the combined spectral peak of Glx to be decreased in adults with cognitively high-functioning autism (Bernardi et al. 2011). The posterior cingulate has not been examined specifically in ASD, but postmortem neuropathological and in vivo imaging studies suggest this area is affected in autism (Oblak et al. 2011).

9.3.5 MRS/MRSI Findings in the Hippocampal–Amygdala Region Across Development in ASD

The hippocampal–amygdala region is the second most frequently part of the brain studied with MRS/MRSI and many other investigative methods. As noted for other regions examined with MRS/MRSI, the findings lack consistency. Neither of the studies in very young children with autism found abnormal concentrations of NAA in this region. All three studies of only older children and adolescents found decreased NAA; however, none of these corrected for partial-volume effects.

Table 9.5 Case–control comparisons of brain metabolites in the frontal lobe[b]

References	Friedman et al. (2003)	Harada et al. (2011)	Chugani et al. (1999)	Hisaoka et al. (2001)	Fujii et al. (2010)	Vasconcelos et al. (2008)
Mean age (years)	3.9	5.2	5.7	5.8	6.1	9.5
Age range (years)	3–4	2–11		2–21	2–13	6–20
Sizes of comparison groups	45 ASD 13 healthy	12 ASD 10 pediatric patients (power = 20 %)	9 autism 5 siblings	55 autism 51 healthy	31 autism 28 pediatric patients	10 autism 10 healthy
Magn. field (T)	1.5	3.0	1.5	1.5	1.5	1.5
Imaging type	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS	SV ^{1}H MRS	SV ^{1}H MRS	SV ^{1}H MRS
Volume/region of interest	Frontal white matter	Lt frontal lobe	Frontal lobe	Bilat frontal lobe	Bilat dorsolateral prefrontal cortex	Lt frontal lobe
Partialvolume effects corrected	Corrected	Corrected	No	No	No	No
Metabolites examined	NAA, Cr, Cho, mI	NAA, Cr, Cho, mI, Glu, GABA	NAA, Lac^{-}	NAA	NAA, Cr, Cho, NAA/Cr Cho/Cr	NAA, Cr, Cho, mI, NAA/Cr NAA/Cho Cho/Cr mI/Cr
Results						
NAA	**– NAA** Lt (also in DD vs. TD)	No difference	No difference	No difference	**– NAA/Cr** Lt	No difference
Cr	**– Cr** left (also in DD vs. TD)	No difference	–	–	No difference	No difference
Cho	No difference	No difference	–	–	No difference	No difference
mI	No difference	No difference	–	–	–	No difference
Lac^{-}	–	–	**+ Lac–** 1 child w autism	–	–	–
Glx (Glu + Gln)	–	–	–	–	–	–
Glu	–	No difference	–	–	–	–
GABA	–	**– GABA**	–	–	–	–

[a]α-nucleotide peaks = combined area for α-peaks of (nucleoside di- and triphosphate groups + dinucleotide and diphosphosugar phosphate groups)

[b]Bold type indicates significant difference between ASD and comparison groups

DeVito et al. (2007)	Levitt et al. (2003)	Kubas et al. (2012)	Endo et al. (2007)	Minshew et al. (1993)	Oner et al. (2007)	Kleinhans et al. (2007)	Murphy et al. (2002)
9.8	10.4	10.5	12.9	20.2	24.3	24.5	30
6–17	5–16	8–15	6–20	Adolescents and adults	17–38	15–44	Adults
26 autism 29 healthy	22 high-functioning ASD 20 healthy	12 autism 16 pediatric	12 autism 15 Asperger 11 PDD 16 healthy	11 autism 11 healthy	14 Asperger 21 healthy	13 high-functioning ASD 13 healthy	14 Asperger 18 healthy
3.0	1.5	1.5	1.5	1.5	1.5	1.5	1.5
2-D ^{1}H MRSI	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS	Surface coils ^{31}P	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS
Frontal gray matter	Frontal cortex, frontal white matter	Lt Rt frontal lobes	Rt prefrontal	Bilat dorsolateral prefrontal region	Rt dorsolateral prefrontal cortex	Lt middle frontal	Rt medial prefrontal region
Corrected	Corrected	No	No	Corrected	No	Corrected	Corrected
NAA, Cr, Cho, mI, Glx No hemispheric differences, so Lt + Rt pooled	NAA, Cr, Cho	NAA/Cr Cho/Cr mI/Cr Glx/Cr GABA/Cr No hemispheric difference	NAA/Cr Cho/Cr	PCr; α-nucleotide[a]; PME, Pi, PDE, ionized phosphate groups, pH	NAA/Cr NAA/Cho Cho/Cr	NAA	NAA, Cr, Cho
– NAA	No difference	**– NAA/Cr**	No difference	–	No difference	**– NAA**	**+ NAA**
No difference	No difference	No difference	–	**– PCr** and esterified ends	–	–	**+ Cr**
No difference	No difference	No difference	No difference	–	No difference	–	**+ Cho**
No difference	–	**+ mI/Cr**	–	–	–	–	–
–	–	–	–	–	–	–	–
– Glx	–	**– Glx/Cr**	–	–	–	–	–
–	–	–	–	–	–	–	–
–	–	**– GABA/Cr**	–	–	–	–	–

Table 9.6 Summary of NAA studies in ASD by level of analysis from global to regional across development (with mean age of participants in the studies indicated)

	NAA	Mean age (years)	Reference
Frontal lobe			
Left	No difference	5.2	Harada et al. (2011)
	No difference	5.8	Harada et al. (2011)
	No difference	9.5	Vasconcelos et al. (2008)
	Decreased (NAA/Cr)	10.5	Kubas et al. (2012)
Bilateral	No difference	5.7	Chugani et al. (1999)
Right	No difference	5.8	Harada et al. (2011)
White matter	Decreased	4.0	Friedman et al. (2003)
	No difference	10.4	Levitt et al. (2003)
Gray matter	Decreased	9.8	DeVito et al. (2007)
	No difference	10.4	Levitt et al. (2003)
Left middle frontal	Decreased	24.5	Kleinhans et al. (2007)
Prefrontal			
Left	No difference	12.9	Endo et al. (2007)
Right	No difference	24.3	Öner et al. (2009)
Dorsolateral prefrontal	Decreased (NAA/Cr)	9.8	Murphy et al. (2002)
Medial prefrontal	Increased	9.8	Fujii et al. (2010)

A fourth study that had the widest age range of individuals (36 years) with improved methodology focused specifically on Asperger disorder and found increased NAA in younger participants but not adults. None of the 3 studies of adults only, all of which performed partial-volume correction, found ASD-control differences in NAA. In contrast, increases in Cr were only found in studies of adults (two of three studies). Decreased Cho was found in very young children with autism, but increased Cho was present in 2 of 6 studies of older children or adults. One report of Glx in the hippocampal–amygdala region in adults found it to be increased. Table 9.8 summarizes the MRS/MRSI studies of the hippocampal–amygdala region in autism.

Nacewicz et al. (2012) highlight a major problem in MRS/MRSI brain studies: it is very difficult to measure biochemical concentrations in small complex structures, like the amygdala, that share boundaries with other structures (e.g., the hippocampus). The need to measure their chemical concentration separately becomes of utmost importance when the two structures respond in different ways to causal risk factors or are differentially affected in particular diseases and disorders. MRS/MRSI studies to date have not been able to measure chemical concentrations in the amygdala separately. The hippocampal–amygdala region has been measured as a whole, except for a recent study that measured the hippocampus (extending into the parahippocampal gyrus) separate from the amygdala. Nacewicz et al. (2012) have focused on reliably measuring chemical concentration ratios in the amygdala separate from the hippocampus by using 1H SV MRS with the addition of outer-volume suppression bands to destroy the MRS signal from the hippocampal regions intruding into the MRS voxel. Their study reveals the impacts of skull air space (internal auditory canal) and neuron-dense microstructures on selecting the optimal MRS

Table 9.7 Case–control comparisons of brain metabolites in cingulate[a]

References	Friedman et al. (2003)	Hisaoka et al. (2001)	Fujii et al. (2010)	Vasconcelos et al. (2008)	Levitt et al. (2003)	Öner et al. (2009))	Bernardi et al. (2011)
Mean age (years)	3.9	5.8	6.1	9.5	10.4	24.3	29.2
Age range (years)	3–4	2–21	2–13	6–20	5–16	17–38	≥18
Sizes of comparison groups	45 ASD 13 healthy	55 autism 51 healthy	31 autism 28 pediatric	10 autism 10 healthy	22 high-functioning ASD 20 healthy	14 Asperger 21 healthy	14 high-functioning ASD 14 healthy
Magn. field (T)	1.5	1.5	1.5	1.5	1.5	1.5	3.0
Imaging type	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS	SV ^{1}H MRS	2-D ^{1}H MRSI	2-D ^{1}H MRSI	2-D ^{1}H MRSI
Volume/region of interest	Cingulate Bilat	Cingulate (Bilat VOIs)	Anterior cingulate (Bilat VOIs)	Anterior cingulate (Bilat VOIs)	Anterior cingulate Lt and Rt	Rt anterior cingulate	Anterior cingulate Lt and Rt
Partial-volume effects corrected	Corrected	No	No	No	Corrected	No	No
Metabolites examined	NAA, Cr, Cho, mI	NAA	NAA, Cr, Cho, NAA/Cr Cho/Cr	NAA, Cr, Cho, mI, NAA/Cr, NAA/Cho, Cho/Cr, mI/Cr	NAA, Cr, Cho	NAA/Cr NAA/Cho Cho/Cr	NAA, Cr, Cho, mI, Glx (average of Rt and Lt)
Results							
NAA	**– NAA** Bilat	No difference	**– NAA/Cr** **– NAA/Cho**	No difference	No difference	No difference	No difference
Cr	No difference	–	No difference	No difference	No difference	–	No difference
Cho	No difference	–	No difference	**+ Cho**	**- Cho** Lt ACC	No difference	No difference
mI	No difference	–	–	**+ mI** **+ mI**/Cr	–	–	No difference
Lac^{-}	–	–	–	–	–	–	–
Glx (Glu + Gln)	–	–	–	–	–	–	**– Glx** Rt ACC

[a]Bold type indicates significant difference between ASD and comparison groups

Table 9.8 Case–control comparisons of brain metabolites in medial temporal lobe: hippocampus and amygdala[a]

References	Zeegers et al. (2007)	Friedman et al. (2003)	Otsuka et al. (1999)	Gabis et al. (2008)	Endo et al. (2007)	O'Brien et al. (2010)	Suzuki et al. (2010)	Kleinhans et al. (2009)	Page et al. (2006)
Mean age (years)	3.6	3.9	8	10.2	12	23	22	24.5	35.6
Age range (years)	2–6	3–4	2–18	7–16	6–20	10–46	19–24	≥18	≥18
Sizes of comparison groups	25 autism 12 MR/LD	45 ASD 13 TD	27 autism	13 high-functioning ASD 8 TD	12 autism 15 Asperger 11 PDD-NOS 16 TD	22 Asperger 22 TD	12 high-functioning autism 12 TD	20 high-functioning ASD 19 TD	25 high-functioning ASD 21 TD
Magn. field (T)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Imaging type	SV ^{1}H MRS	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS	SV ^{1}H MRS	^{1} SV ^{1}H MRS	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS
Volume/region of interest	Lt hippocampus–amygdala region	Lt and Rt medial temporal lobe	Rt hippocampus–amygdala region	Lt and Rt hippocampus–amygdala regions	Rt medial temporal lobe: amygdala and part of hippocampus	Rt amygdala–hipocampus	Lt hippocampus with some parahippocampus	Bilat amygdala often extending into hippocampal region	Hippocampus–amygdala region
Partial-volume effects corrected	No	Corrected	No	No	No	Corrected	Corrected	Corrected	Corrected
Metabolites examined	NAA, Cho, Cr, NAA/Cho, Lac^{-}	NAA, Cr, Cho, mI	NAA	NAA/Cr Cho/Cr mI/Cr	NAA/Cr Cho/Cr	NAA, Cr, Cho, mI NAA/Cr, Cho/Cr	NAA, Cr, Cho	NAA, Cr, Cho, mI average of Rt and Lt	NAA, Cr, Cho, mI, Glx

Results									
NAA	No difference	No difference	**– NAA**	**– NAA/Cr** Bilat	**– NAA/Cr** in autism vs. PDD and TD **– NAA/Cr** Asperger vs. TD	**+ NAA** only in younger Asperger (10–16 years) NAA and NAA/Cho: abnormal age trajectory in Asperger (decreasing)	No difference	No difference	No difference
Cr	No difference	No difference	–	–	–	No difference	**+ Cr**	No difference	**+ Cr**
Cho	No difference	**– Cho Rt** **+ Cho T2 Rt**	–	**+ Cho/Cr** Lt	No difference	Cho: abnormal age trajectory in Asperger (decreasing)	**+ Cho**	No difference	No difference
mI	–	Rt. + Cho **T2**	–	**+ mI/Cr** Bilat	–	–	–	No difference	No difference
Lac^{-}	No difference	–	–	–	–	–	–	–	–
Glx	–	–	–	–	–	–	–	–	**+ Glx**

[a]Bold type indicates significant difference between ASD and comparison groups

Table 9.9 Case–control comparisons of brain metabolites in thalamus[a]

References	Friedman et al. (2003)	Levitt et al. (2003)	Hardan et al. (2008)	Bernardi et al. (2011)
Mean age (years)	3.9	10.4	11.9	29.2
Age range (years)	3–4	5–16	8–15	≥18
Sizes of comparison groups	45 ASD 13 healthy	22 high-functioning ASD 20 healthy	18 high-functioning autism 18 healthy	14 high-functioning ASD 14 healthy
Magn. field (T)	1.5	1.5	1.5	3.0
Imaging type	22-D ^{1}H MRSI	2-D ^{1}H MRSI	2-D ^{1}H MRSI	2-D ^{1}H MRSI
Volume/region of interest	Bilat thalamus	Bilat thalamus	Bilat thalamus	Bilat thalamus
Partial-volume effects corrected	Corrected	Corrected	No	No
Metabolites examined	NAA, Cr, Cho, mI	NAA, Cr, Cho	NAA, Cr, Cho	NAA, Cr, Cho, mI, Glx
Results				
NAA	**– NAA**, **+ NAA T2** Rt thalamus	No difference	**– NAA** Lt thalamus	No difference
Cr	**– Cr** Lt thalamus	No difference	**– Cr** Lt thalamus	No difference
Cho	**– Cho** Lt thalamus	No difference	**– Cho** Lt thalamus	No difference
mI	No difference	–	–	No difference
Lac^{-}	–	–	–	–
Glx	–	–	–	No difference

[a]Bold type indicates significant difference between ASD and comparison groups

volume for sampling the amygdala and demonstrates an elegant technique they intend to use to study developmental changes in the amygdala in typical development, autism, and other disorders.

9.3.6 MRS/MRSI Findings in the Thalamus Across Development in ASD

Table 9.9 summarizes the results of the small number of MRS/MRSI studies that have examined thalamic metabolite concentrations in ASD. The thalamus had the greatest number of abnormalities specific to ASD in a methodologically high-quality MRSI study of very young children (Friedman et al. 2003). The young children in that study were diverse in regard to general cognitive ability; some were high functioning, but many were low functioning with some degree of intellectual handicap (MR). Three other studies examined the thalamus with MRSI, but only in cognitively higher-functioning children and adults with ASD. All of the abnormalities found in the young children were replicated in one of the two studies of older children but not in a study of adults. The combined results suggest that the thalamus

is biochemically abnormal early in childhood development in autism, and although the abnormalities can persist into later childhood and early adolescence in some cases, they appear to resolve by adulthood. The validity of the trend needs to be tested with replication studies and particularly longitudinal investigations, measuring changes over time within the same individuals. In contrast to studies of other brain regions that found abnormalities of Glx in adults with ASD, no case–control differences were found in the single study of the thalamus in adults with autism.

9.3.7 MRS/MRSI Findings in the Cerebellum Across the Development in ASD

To date, there are no published studies of chemical concentrations in the cerebellum in very young children with ASD. Of the five studies in older children and two studies in adults, described in Table 9.10, only two studies found significantly decreased concentrations of NAA. Differences in Cr and Cho were found in only one study. A preliminary but important finding, from a study with sizeable cohorts, is an 11.7 % reduction in Glx in children with autism who are 6 to 17 years of age (DeVito et al. 2007).

9.3.8 Age-Related Changes in Metabolites: Are They the Same in ASD and Typical Development?

Inferences from cross-sectional data about developmental processes must be made with caution (Kraemer 2003). The inferences need to be confirmed with longitudinal studies measuring change with age within individuals. Cross-sectional studies can suggest important developmental mechanisms and generate hypotheses for longitudinal studies.

Even in typical development, most postnatal developmental changes in metabolite concentrations during childhood and adolescence are inferred from cross-sectional data. The National Institutes of Health (NIH) MRI Study of Normal Brain Development has collected MRS data longitudinally from birth; results and data release are forthcoming (http://pediatricmri.nih.gov/nihpd/info/project_overview.html). Cross-sectional studies published to date show that NAA increases markedly during the first year of life, increases to different degrees in different brain regions during the following 1–2 years, and slightly increases during later childhood and adolescence and plateaus in early adulthood (van der Knaap et al. 1990; Kreis et al. 1993, 2002). It subsequently decreases in adulthood, as demonstrated in recent studies of adults from 18 to 84 years of age (Maudsley et al. 2009, 2012). Cr also increases after birth, although not as dramatically as NAA. Cr appears to peak at about 6 years of age and is thought to then remain quite stable into young adulthood. Because of its stability in typical development, it has often been used as an internal reference in the denominator of metabolite concentrations. However, it should be

Table 9.10 Case–control comparisons of brain metabolites in the cerebellum[a]

	Chugani et al. (1999)	Vasconcelos et al. (2008)	DeVito et al. (2007)	Gabis et al. (2008)	Endo et al. (2007)	Suzuki et al. (2010)	Kleinhans et al. (2007)
Mean age (years)	5.7	9.5	9.8	10.2	12.9	22	24.5
Age range (years)	–	6–20	6–17	7–16	6–20	19–24	15–44
Sizes of comparison groups	9 autism 5 siblings	10 autism 10 healthy	26 autism 29 healthy	13 high-functioning ASD 8 healthy	12 autism 15 Asperger 11 PDD 16 healthy	12 high-functioning autism 12 healthy	13 high-functioning ASD 13 healthy
Magn. field (T)	1.5	1.5	3.0	1.5	1.5	1.5	1.5
Imaging type	SV ^{1}H MRS	SV ^{1}H MRS	2-D ^{1}H MRSI	SV ^{1}H MRS	SV ^{1}H MRS	2-D ^{1}H MRSI	SV ^{1}H MRS
Volume/region of interest	Cerebellum	Lt cerebellar hemisphere	Cerebellum	Cerebellum	Cerebellar vermis	Rt cerebellum	Rt cerebellar hemisphere Midline vermis
Partial-volume effects corrected	No	No	Corrected	No	No	Corrected	Corrected
Metabolites Examined	NAA, Lac^{-}	NAA, Cr, Cho, mI, NAA/Cr, NAA/Cho, Cho/Cr, mI/Cre	NAA, Cr, Cho, mI, Glx No hemispheric difference, so Lt + Rt pooled	NAA/Cr Cho/Cr mI/Cr	NAA/Cr Cho/Cr	NAA, Cr, Cho	NAA
Results							
NAA	**– NAA**	No difference	No difference	No difference	No difference	**– NAA**	No difference
Cr	–	No difference	No difference	**–**	**–**	No difference	–
Cho	–	No difference	No difference	**+ Cho/Cr**	No difference	No difference	–
mI	–	No difference	No difference	**+ mI/Cr**	–	–	–
Lac^{-}	No difference	–		–	–	–	–
Glx	–	–	**– Glx**	–	–	–	–
GABA	–	–	–	–	–	–	–

[a]Bold type indicates significant difference between ASD and comparison groups

Table 9.11 Summary of cross-sectional case–control comparison and age-related changes in the right hippocampal–amygdala complex in Asperger disorder and typically developing groups

	Case–control comparison of means		Cross-sectional changes with age, from 10 to 46 years	
	Asperger vs. TD			
	$N=22$	$N=22$	TD sample	Asperger sample
Volume of right amygdala				
Raw whole volume	No difference		Similar age-related changes	
Volume normalized to total brain volume	No difference			
Volume of right hippocampus				
Raw whole volume	No difference		Linear decrease with age	No change with age
Volume normalized to total brain volume	No difference		Curvilinear decrease until age 20 years	No change with age
MRS right hippocampal–amygdala complex				
NAA	No difference		No change with age	Declining
NAA/Cr	No difference		Slight increase with age	Declining
Cho	No difference		No change with age	Declining

noted that equivalent stability in Cr concentrations may not hold in brains with neurodevelopmental disorders, and even typically developing brain may display differences in Cr between different brain regions (Nacewicz et al. 2012). Cho and mI decrease after birth, plateauing in early adulthood and childhood, respectively (Kreis et al. 1993). From 18 years of age to late adulthood, Cr and Cho increase in measured concentration (Maudsley et al. 2009).

The MRS study of ASD by O'Brien et al. (2010) purposefully included participants with a wide age range, in order to examine cross-sectional age-related changes in metabolites and volume in the right amygdala–hippocampal complex. The investigators compared 22 individuals with Asperger disorder and 22 TD controls 10 to 46 years of age. (Criteria for Asperger disorder included meeting autistic criteria for social impairment and ritualistic behavior with no early language delay and no intellectual subnormality.) Although there were no group differences in amygdala volume, hippocampal volume, or mean metabolite concentrations, age-related changes in hippocampal volume and metabolites in the amygdala–hippocampal regions were atypical in the Asperger group. The results are summarized in Table 9.11. In the TD group, hippocampal volume was decreasing with age, while NAA and Cho remained unchanged. In contrast, hippocampal volume did not decrease as expected in the Asperger group, and NAA, NAA/Cr, and Cho abnormally decreased with age. No differences from the TD sample were found in cross-sectional age-related changes in concentrations of Cr or mI.

The investigators observed what is often found in case–control studies of neurodevelopmental psychiatric disorders: age-dependent differences can be obscured by whole-group mean comparisons. The results provide additional evidence of complex neurodevelopmental abnormalities in the amygdala–hippocampal region in ASD.

Table 9.12 Differences in metabolite concentrations in healthy male and female adults

	Males (relative to females)		
	NAA	Cr	Cho
Frontal lobe			
Gray matter	No difference	No difference	No difference
White matter	Decreased	Decreased	Decreased
Temporal lobe			
Gray matter	Decreased	Decreased	Decreased
White matter	Decreased	Decreased	Decreased
Parietal lobe			
Gray matter	No difference	No difference	No difference
White matter	No difference	No difference	No difference
Occipital lobe			
Gray matter	Decreased	Decreased	Decreased
White matter	Increased	Increased	Increased
Cerebellum	No difference	No difference	No difference

They highlight the need to further develop reliable MRS/MRSI methods to examine metabolites in the amygdala and hippocampus separately. The results demonstrate that studying ASD with multiple types of neuroimaging, such as volumetric MRI and MRS in the O'Brien et al. (2010) study, results in scientifically richer information that suggests differential mechanisms involved in brain development and maturation in ASD. The results highlight the critical need for reliable longitudinal multimodal imaging studies with sufficient sample sizes of ASD and comparison individuals to tease apart and help understand what appear to be complex mechanisms involved in brain development and maturation in individuals with ASDs. An indication of the sample sizes that will be required is available from Steen et al.'s (2005) systematic review of the more extensive MRS literature on schizophrenic subjects and controls, wherein they estimate that minimum sample sizes of 39 patients and 39 controls would be required to detect a 10 % difference in NAA with 80 % power. To detect a 5 % difference with 80 % power, the minimum sample sizes for patient and control groups rise to 130 in each cohort. These estimates are probably valid for 1H SV MRS studies in ASD patients as well.

9.3.9 Metabolite Concentrations in Males and Females: Do They Differ in Similar Ways in ASD and Typical Development?

Metabolite measures in 63 healthy males and 77 healthy females have been compared at the lobar level (left and right frontal, temporal, parietal, and occipital lobes) and in the cerebellum (Maudsley et al. 2012). Males and females had significantly different metabolite levels in frontal lobe white matter, temporal lobe gray and white matter, and occipital lobe gray and white matter. Table 9.12 summarizes these findings.

The NIH MRI Study of Normal Brain Development will provide important information about male–female differences and similarities in metabolites in children. Until then and after more females with ASD are studied, the important question about male–female differences in metabolites in ASD cannot be answered. Extremely few females with ASD have been studied with MRS/MRSI; most studies include no females and studies including females usually include only 1 or 2. The study by Friedman et al. (Friedman et al. 2003) that focused on a relatively large sample of 3–4-year-old children (ASD: 7 girls, 38 boys; DD: 9 girls, 6 boys; TD: 2 girls, 11 boys) tested sex as a covariate and found no effect.

9.3.10 Other Factors Affecting Measured Metabolite Levels in Adults

Body mass index (BMI) affects the measure of metabolites in some regions of the brain (occipital and parietal lobes and cerebellum). BMI also affects a number of factors associated with the quality of MRS data acquisition and analysis, including B0 inhomogeneity, signal-to-noise ratio, and spectral line width (Maudsley et al. 2012). Other factors in adults influencing metabolite signals are smoking, alcohol consumption, and hypertension. In addition, other neurological and neuropsychiatric conditions that might emerge in individuals with autism, such as major depression, OCD, and seizures, are associated with alterations of metabolite concentrations.

9.3.11 Psychotropic Medications, Sedation for Scanning, and Metabolite Concentrations

Many individuals with an ASD are treated with at least one psychotropic medication. These medications often improve associated symptoms and conditions, such as overactivity, inattention, irritability, and mood and anxiety disorders that add significant additional impairment to that caused by the core diagnostic features of ASD. Unless otherwise indicated in Table 9.13, some of the ASD participants in the MRS/MRSI studies were taking psychotropic medications. Exceptions include studies of very young children with ASD, most of whom were medication naïve, and some studies of older individuals that excluded ASD participants who were taking psychotropic medications.

Two studies of children and adolescents with ASD examined the effect of psychotropic medication use on case–control comparisons of brain metabolites (Levitt et al. 2003; DeVito et al. 2007). After comparing the control samples to the total ASD samples, both investigators divided their ASD samples into two subgroups: individuals with autism who were being treated with one or more psychotropic medications and those who were not being treated. Levitt et al. (2003) reran *t*-tests after excluding children in the autism sample who were being treated with

Table 9.13 Summary of published MRS/MRSI studies of autism to date

References	ASD sample size	Control sample size	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Magn. field (T)	MRS study type PVC: partial-volume correction[a]	Brain region	Findings in ASD compared to controls
Minshew et al. (1993)	11M	11M	Adolescents and adults	Autism (ADI, ADOS, DSM-III; all IQ >70)	Healthy controls	1.5	Surface coils, ^{31}P PVC	Bilat dorsal prefrontal cortex	PCr; α-nucleotide; PME, Pi, PDE, ionized phosphate groups, pH: no difference Decreased neuropsychological test performance was associated with decrease in highest energy phosphate compounds and membrane building blocks and with increased levels of membrane breakdown products
Hashimoto et al. (1998)	28 20M, 8F		Children 5.6 ± 2.2 2–12	Autism	–	1.5	SV ^{1}H MRS	Rt parietal lobe	NAA/Cho, NAA/Cr, Cho/Cr No difference
Chugani et al. (1999)	9 8M, 1F	5 4M, 1F	Children 5.75 ± 2.5 3–12	Autism (DSM-IV, CARS)	Siblings of the autism children	1.5	SV ^{1}H MRS	Frontal lobe Temporal lobe Cerebellum	NAA: reduced in cerebellum Lac^{-}: increased in one child in frontal lobe
Otsuka et al. (1999)	27 21M, 6F		Children 2–18	Autism	–	1.5	SV ^{1}H MRS	Rt hippocampus–amygdala Lt cerebellum	NAA: reduced in Rt hippocampus–amygdala and Lt cerebellum
Hisaoka et al. (2001)	55 47M, 8F	51 26M, 25F	Children 5.8 2–21	Autism (ICD-10)	Healthy controls	1.5	SV ^{1}H MRS	Bilat frontal lobe Temporal lobe Parietal lobe Cingulate Brain stem	NAA: reduced in Bilat temporal lobe
Murphy et al. (2002)	14M	18M	Adults 30 ± 9	Asperger (ADI, ICD-10; no meds at time of study)	Healthy controls	1.5	SV ^{1}H MRS PVC	Rt medial Prefrontal Rt parietal	NAA, Cho, Cr: all increased in Rt medial prefrontal

Sokol et al. (2002)	10 9M, 1F	0	5.3 2.5–12	Autism (DSM-IV; 3 had seizures, 6 had MRI abnormal)	–	1.5	SV ^{1}H MRS	Mid-temporal lobe within region of hippocampus	Cho/Cr was significantly correlated with Childhood Autism Rating Scale score
Strauss et al. (2002)	21	13	6–15	ASD treated with fluvoxamine or fluoxetine	Non-ASD adults treated with fluvoxamine or fluoxetine	1.5	^{19}F (fluorine MRS)	Whole brain	Brain concentrations of fluoxetine/fluvoxamine relative to a phantom ASD children treated with fluoxetine/fluvoxamine compared to non-ASD adults with panic disorder, major depression, or OCD treated with fluoxetine/fluvoxamine Results Both groups: drug dose and brain drug concentration related; no significant differences in drug concentrations between children and adults after correction for dose/body mass
Friedman et al. (2003)	45 38M, 7F	28 17M, 11F	Young children 3.9 ± 0.4 3–4	ASD (ADI, ADOS; propofol for scanning)	13 healthy controls; 15 DD	1.5	2-D ^{1}H MRSI PVC	21 brain regions	NAA, Cr, mI: widespread regional reductions NAA T2 relaxation: prolonged
Levitt et al. (2003)	22 18M, 4F	20	Children 10.4 ± 3.4 5–16	ASD (ADI, ADOS, DSM-IV; 21 had IQ ≥ 70)	Healthy controls	1.5	2-D ^{1}H MRSI PVC	22 brain regions	NAA: no difference Cho: reduced Lt anterior cingulate; increased Rt caudate Cr: reduced Lt caudate and Rt occipital; increased Rt caudate

(continued)

Table 9.13 (continued)

References	ASD sample size	Control sample size	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Magn. field (T)	MRS study type PVC: partial-volume correction[a]	Brain region	Findings in ASD compared to controls
Fayed and Modrego (2005)	21 18M, 3F	20 8M, 12F	Children 7.3 ± 4.1	Autism (DSM-IV; 5 had IQ < 80)	12 healthy controls 8 ADHD	1.5	SV ^{1}H MRS PVC	Lt centrum semiovale	NAA/Cr, Cho/Cr, mI/Cr: No autism-typical control differences Higher NAA/Cr in ADHD than in autism or typical controls
Page et al. (2006)	25 20M, 5F	21	Adults 35.6 ± 11.5	ASD (ICD-10, ADI; FSIQ > 70)	Healthy controls	1.5	SV ^{1}H MRS	Rt amygdala–hippocampal region Rt parietal lobe	NAA, Cho, mI: no group differences Glx, Cr + PCr: both increased in Rt hippocampus–amygdala
Friedman et al. (2006)	45 38M, 7F	22 13M, 9F	Young children 4.0 ± 0.4 3–4 years	ASD (ADI, ADOS; propofol for scanning)	10 healthy controls; 12 DD	1.5	2-D ^{1}H MRSI PVC	21 brain regions: gray matter compared to white matter	NAA, Cho, Cr, mI: reductions predominate in gray matter compared to white matter Cho T2 relaxation: prolonged in gray matter Glx, Lac^{-}: no case–control differences
Zeegers et al. (2007)	25M	12	Young children AD: 3.6 ± 0.6 PDD: 3.8 ± 1.3 2–6	ASD (ADI, ADOS)	MR/LD	1.5	SV ^{1}H MRS	Lt frontal subcortical white matter Lt hippocampus–amygdala	NAA, Cho, Cr, NAA/Cr, NAA/Cho, Lac^{-}: no differences
Kleinhans et al. (2007)	13M	13	Adolescents, adults 24.5 ± 9.5 15–44	ASD (ADI, ADOS, DSM-IV, FSIQ > 80)	Healthy controls	1.5	SV ^{1}H MRS PVC	Lt middle frontal gyrus Lt superior parietal and occipital cortex Rt cerebellar hemisphere Midline vermis	NAA: reduced in combined regions and in Lt middle frontal gyrus

Endo et al. (2007)	38 32M, 6F	16	Children, adolescents 12.9 ± 3.8 6–20	Autism $n = 12$; AD $n = 15$, PDD-NOS $n = 11$ (DSM-IV)	Healthy controls	1.5	SV ^{1}H MRS	Rt prefrontal Rt medial temporal Cerebellar vermis	NAA/Cr: reduced in Rt medial temporal in autism compared to PDD and controls and in Asperger compared to controls Cho/Cr: no group difference
DeVito et al. (2007)	26M	29M	Children, adolescents 9.8 ± 3.2 6–17	Autism (ADI, ADOS, DSM-IV; pIQ > 70)	Healthy controls	3.0	2-D ^{1}H MRSI PVC	Cerebral GM Cerebral WM Frontal GM Temporal GM Occipital GM Cerebral WM Cerebellum	Cr, Cho, mI: no group differences NAA, Glx: widespread reduction in cerebral gray matter and cerebellum
Oner et al. (2007)	14M	M	Adults 24.3 ± 7.1 17–38	Asperger (DSM-IV)	Healthy controls	1.5	2-D ^{1}H MRSI	Rt dorsolateral prefrontal and Rt anterior cingulate cortices (not reported: Rt amygdala)	NAA/Cr, Cho/Cr: no differences NAA/Cho: increased in Rt anterior cingulate
Vasconcelos et al. (2008)	10M	10M	Children 9.53 ± 1.8 (median age)	Autism (DSM-IV; 9 received sevoflurane for scanning)	Healthy controls	1.5	SV ^{1}H MRS	Anterior cingulate Lt frontal lobe Lt striatum Lt cerebellar hemisphere	NAA, Cr, NAA/Cr, Cho/Cr, NAA/Cho: no group differences Cho, mI, mI/Cr: increased in anterior cingulate mI/Cr: increased in Lt striatum
Gabis et al. (2008)	13 10M, 3F	8 3M, 5F	Children, adolescents 10 ± 2.5 7–16	ASD (DSM-IV; pIQ or vIQ ≥ 80); no regular medications; no sedation for scanning	Healthy controls	1.5	SV ^{1}H MRS	Bilat hippocampus–amygdala Cerebellum	NAA/Cr: reduced in Bilat hippocampus–amygdala Cho/Cr: increased in Lt hippocampus–amygdala and cerebellum mI/Cr: increased in Bilat hippocampus–amygdala and cerebellum

(continued)

Table 9.13 (continued)

References	ASD sample size	Control sample size	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Magn. field (T)	MRS study type PVC: partial-volume correction[a]	Brain region	Findings in ASD compared to controls
Hardan et al. (2008)	18M	16M	Children 11.9 ± 2.2 8–15	Autism (ADI, ADOS; FSIQ > 70)	Healthy controls	1.5	2-D ^{1}H MRSI	Bilat thalamus (additional regions: data not reported)	NAA, Cr, Cho: reduced in Lt thalamus
Kleinhans et al. (2009)	20 18M, 2F	19 17M, 2F	Adults	ASD (ADI, ADOS, DSM-IV; all had FSIQ ≥ 80)	Healthy controls	1.5	SV ^{1}H MRS PVC	Averaged Lt + Rt amygdala–hippocampus	NAA, Cr, Cho, mI in average of Lt and Rt amygdala–hippocampus: no differences
Endo et al. (2010)	26 21M, 5F	–	Children, adolescents 13.4 ± 3.7; 8–20	ASD (DSM-IV; 16 S/S and 10 S/L for *5-HTTLPR*)	–	1.5	SV ^{1}H MRS	Rt medial prefrontal cortex Rt medial temporal lobe Cerebellar vermis	NAA/Cr decreased in Rt medial prefrontal cortex in ASD participants with S/S compared to S/L genotype of *5-HTTLPR* polymorphism Cho/Cre: no difference
Fujii et al. (2010)	31 25M, 6F	28 22M, 6F	Children 6.1 2–13	Autism (DSM-IV; 27 had IQ < 70)	Pediatric patients with negative exam	1.5	SV ^{1}H MRS	Anterior cingulate cortex (ACC) Bilat dorsolateral prefrontal cortex (DLPFC); 20 autism, 18 controls)	NAA, Cr, Cho, Cho/Cr: no group differences NAA/Cr: reduced in ACC and Lt DLPFC NAA/Cho: reduced in ACC

O'Brien et al. (2010)	22 18M, 4F	22 20M, 2F	Children, adults 23 ± 13 10–46	Asperger disorder (ADI or ADOS, ICD-10)	Healthy controls	1.5	SV ^{1}H MRS PVC	Rt amygdala–hippocampus	Glx: excluded due to lack of significant model fit NAA, Cr, Cho, mI and ratios: no total group differences Younger AS patients (10–16 years) but not older patients (20–46 years) had higher NAA and NAA/Cr than age-matched controls Group differences in cross-sectional age-related changes in NAA, NAA/Cr, and Cho: decrease with age in Asperger but not in controls Suggests differences in amygdala–hippocampal maturation and neuronal and lipid membrane integrity
Suzuki et al. (2010)	12M	12M	Adults 22 ± 1.8 19–24	autism (ADI, ADOS; all IQ ≥ 70); all aggressive but on no medications	Healthy controls	1.5	2-D ^{1}H MRSI PVC	Lt hippocampus Rt cerebellum	NAA: reduced in cerebellum Cr and Cho: increased in hippocampus
Harada et al. (2011)	12	10	Children 5.2 ± 3.0 2–11	ASD (DSM-IV) (n = 10 triclofos sodium for scanning)	Pediatric patients with negative exam (9 sedated)	3.0	SV ^{1}H MRS PVC	Lt frontal lobe Lt lenticular nucleus	NAA, Cr, Cho, mI, Glu: no differences, but power < 20 % GABA: reduced in Lt frontal lobe

(continued)

Table 9.13 (continued)

References	ASD sample size	Control sample size	ASD age in years (mean ± SD; range)	ASD diagnosis	Controls	Magn. field (T)	MRS study type PVC: partial-volume correction[a]	Brain region	Findings in ASD compared to controls
Bernardi et al. (2011)	14 12M, 2F	14 11M, 3F	Adults 29.2 ± 6.1	High-functioning ASD (ADI-R, ADOS, DSM-IV; IQ ≥ 80; no psychotropic medications)	Healthy controls	3.0	2-D ^{1}H MRSI	Bilat anterior cingulate Thalamus Intraparietal sulcus Temporoparietal junction (other regions not reported)	NAA, Cr, Cho, mI, Glx: Glx was reduced in Rt anterior cingulate mI was reduced in the Lt temporoparietal junction
Corrigan et al. (2011)	1M (case report)	7M	63	Autistic savant (prodigious artistic skills; also gifted in music, pitch, languages, and sound imitation)	Highly educated healthy controls (mean age 5.8 ± 5.3; 50–66)	3.0	2-D ^{1}H MRSI (for NAA, Cho, Cre) and J-resolved MRS data (for Glu, GABA) from large single-voxel PVC	Three contiguous slabs centered on corpus callosum Large single voxel centered on midline in parietal lobe	NAA, Cho, Cr: no difference (i.e., within 2 standard deviations of control group mean) Glu, GABA: markedly decreased in parietal lobe (i.e., more than 2 standard deviations below control group mean)
Ipser et al. (2012)	465	387	Children, adults	ASD (ADI-R, ADOS, DSM-IV, ICD-10)	Healthy controls	1.5 (17 total) and 3 (3 total)	^{1}H MRS (mixed)	Caudate, cerebellum, cingulated, lenticular nucleus, parietal lobe, putamen, temporal lobe, thalamus	NAA: decreased in children in whole-brain gray and white matter; decreased overall in parietal cortex, cerebellum, temporal lobe Cr: increased in adults in whole-brain gray matter, temporal lobe; decreased in children in occipital lobe NAA, Cr: no difference in frontal lobes

Kubas et al. (2012)	12	16	Children 10.5 ± 4.9 8–15	Autism (ICD-10)	Pediatric patients with negative exam	1.5	SV ^{1}H MRS	Bilat frontal lobes	NAA/Cr, GABA/Cr, Glx/Cr: all were lower in the frontal lobes mI/Cr: increased in the frontal lobes Cho/Cr: no difference No hemispheric differences
Corrigan et al. (2012)	54 38M, 7F	22 DD 54 TD	Longitudinal at ages 3–6, 6–7, and 9–10	ASD (ADI, ADOS, DSM-IV)	DD longitudinal, 55 % healthy controls cross-sectional, 20 %	1.5	2-D ^{1}H MRSI PVC	Whole-slab, gray matter, white matter, ventricular	Brain Lac^{-}: no differences between diagnostic groups at any age point or all age points combined Supports no brain mitochondrial dysfunction in this autism sample

[a]As reported in published manuscript

Not reported in this table or chapter, but potentially important, are several non-English articles on MRS studies of autism (Mori et al. 2001; Perich-Alsina et al. 2002; Tsuda et al. 2008; Öner et al. 2009)

psychotropic medication. The original case–control results remained significant, indicating that psychotropic medications had not influenced the results. In fact, medication use might have decreased the difference between the overall ASD group and controls. The original change was a 21 % increase in Cr in the head of the right caudate nucleus, for the entire autism group. Cr concentration in this region was 24 % lower in the medicated autism subgroup than in the autism subgroup not being treated with medications, making it closer to typical control levels. In the second study, DeVito et al. (2007) performed a three-group ANCOVA: the ASD subgroup being treated with psychotropic medication ($n=12$), the no-medication ASD subgroup ($n=14$), and the healthy control group ($n=29$). The main findings of their study remained significant: compared to TD controls, NAA and Glx were decreased in cerebral gray matter in both ASD subgroups, and the ASD subgroups did not differ from each other. The autism samples and subgroups in both studies were too small to examine the effect of individual types of psychotropic medications. No information is available about potential effects of psychotropic medication use, often of many years duration, on metabolite concentrations in adults with ASD.

A number of MRS/MRSI studies of ASD used sedation for scanning, particularly studies of young children. Different types of administration of sedation (oral and IV) and different sedation medications were used. Levitt et al. (2003) and DeVito et al. (2007) systematically tested for effects of sedation on case–control metabolite differences. Again dividing their autism sample into subgroups, they compared ASD children who had received sedation medications for scanning, those who had not been sedated, and their typical control group. In the Levitt et al. (2003) study that used IV propofol for sedation, all original case–control findings remained significant when children who had been sedated were excluded from the analyses, except for the finding of a 19.1 % increase in Cho in the head of the right caudate. The DeVito et al. (2007) study used oral midazolam for scanning in 18 of the 26 children. The two autism subgroups (sedation and no sedation) and the TD control group were compared. The main findings of their study remained significant: the sedation and sedation-free autism subgroups did not differ from each other and both showed significantly decreased NAA and Glx in cerebral gray matter compared to the TD controls.

The combined results suggest that treatment with psychotropic medications and use of sedation for scanning do not significantly affect case–control comparisons of brain metabolite concentrations in most cases, at least not in global regions, such as gray matter within large volume of interest slabs. But the pattern of psychotropic medications, type and route of sedation for scanning, and depth of sedation (deep sedation vs. anesthesia) varies from study to study. Effects of psychotropic medication and sedation on case–control metabolite quantification results should therefore be tested, when possible, in every study in which they have the potential to influence the results.

9.3.12 Cerebral Volume and Metabolite Measures

The rate of macrocephaly is increased in autism (overall about 20 %). It is present at birth in a minority of cases, but it develops during the first few years of life in

most cases, because of accelerated brain growth. By young adulthood, mean brain size is no different in autism from that in typical development. MRS/MRSI studies of young children with autism will, in particular, often be faced with significantly increased mean total brain volume in the autistic children compared to the control sample.

The developmental mechanisms and neural correlates and consequences of accelerated brain growth and enlarged brain size that occur in some young children with autism are still not known. Friedman et al. (2006), in their study of metabolites in gray matter and white matter in young children with ASD and comparison children, used cerebral volume as a covariate because they found it was associated with their biochemical measures. Cerebral volume was not significant for the absolute concentration of any of the metabolites measured in the young children (NAA, Cr, Cho, mI, Glx, Lac^{-}), but it was significant for T2, transverse relaxation, of NAA and Cr in gray matter and white matter. The findings suggest that the effects of cerebral volume on T2 in the study are due to an underlying pathological process (Friedman et al. 2006).

9.3.13 ASD Subtypes, Differences in Clinical Features, and Metabolite Levels

Although the DSM-V will likely eliminate the ASD subtypes (autistic disorder, Asperger disorder, and PDD-NOS) because they are not reliably distinguished in clinical practice and appear to be part of a more continuous spectrum that varies in severity along a number of dimensions, it is important to note past studies that have examined potential ASD subtype affects on metabolite measures. Friedman et al. (2006) compared significant main effects of ASD in gray matter and white matter (Table 9.4) in autistic disorder ($n=29$) and PDD-NOS ($n=16$) subgroups of their ASD sample. The only difference between the 2 ASD subtypes was a statistical trend in gray matter NAA: the autistic disorder subgroup had slightly lower NAA (2 %) than the PDD-NOS group. Endo et al. (2007) compared NAA/Cr and Cho/Cr ratios in the medial prefrontal cortex, medial temporal lobe, and cerebellar vermis in young people with DSM-IV-diagnosed autistic disorder ($n=12$), Asperger disorder ($n=15$), and PDD-NOS ($n=11$) to each other and to TD controls ($n=16$). NAA/Cr was lower in the medial temporal lobe in the autistic disorder group and the Asperger group compared to TD controls. NAA/Cr was also lower in the autistic disorder group compared to the PDD-NOS group. No other group differences were found. The authors acknowledge that the sample sizes of the ASD subtype cohorts were small and affected the robustness of statistical comparison.

Comparisons to understand individual differences in the clinical manifestations of ASDs are, nevertheless, very important. Several dimensions along which children and adults with ASD vary are IQ, language level, and core social, communication, and stereotyped repetitive features. Kleinhans et al. (2009) examined correlations, controlling for age, between NAA, Cr, Cho, and mI in the hippocampal–amygdala complex (HAC) of 20 adults with cognitively high-functioning ASD and both

ADI-R measures of core features of autism during early childhood and ADOS-G measures of social and communicative features at the time of MRS evaluation. Correlations were found between metabolite concentrations and severity of symptoms of autism during early childhood but not concurrent symptoms: NAA was negatively correlated with ADI-R communication score, NAA and Cr with ADI-R stereotyped behaviors and interests score, and Cr with ADI-R social algorithm score; and more severe autistic signs during early childhood were associated with lower metabolite levels in the hippocampal–amygdala complex in adulthood. Murphy et al. (2002) observed correlations between metabolite levels in the medial prefrontal lobe of adults with Asperger disorder, ADI, and Yale-Brown Obsessive–Compulsive Scale: as prefrontal NAA increased, obsessive–compulsive behavior increased ($r=0.67$, $p=0.005$) and more severely impaired ADI-R communication during early childhood was associated with increased prefrontal Cho in adulthood ($r=0.72$, $p=0.02$).

Small sample sizes in the studies reviewed permitted only preliminary analysis of the relationship between IQ and metabolite levels in TD controls and ASD participants. In healthy controls ($n=20$, age range 6–16 years), Levitt et al. (2003) found that FSIQ was negatively correlated with NAA in the left frontal lobe ($r=-.50$, $p=0.018$). In contrast, in a different healthy control sample ($n=19$), Kleinhans et al. (2009) found average NAA in the combined left and right hippocampal–amygdala complex to be positively correlated with FSIQ. Gabis et al. (2008) observed mI/Cr in left and right hippocampal–amygdala region to be negatively correlated with pIQ, vIQ, and FSIQ ($r=-0.786$ to -0.843, all $p<0.05$) in their control group. In contrast, in their ASD sample, Gabis et al. (2008) found positive correlations between NAA/Cr and mI/Cr in the right hippocampus–amygdala and pIQ ($r=0.674$, $r=0.618$, both $p<0.05$). In a predominantly cognitively low-functioning sample of children with autism, IQ poorly correlated with NAA/Cr in the anterior cingulate and bilateral dorsolateral prefrontal cortex (DLPFC).

Studies in samples of small sizes also suggest possible relationships between metabolite levels and language delay in ASD. In the Gabis et al. (2008) study, 8 of 13 participants with ASD had evidence of a language delay based on child neurology clinical assessment. Hippocampal–amygdala complex (HAC): NAA/Cr ratios in the left and right HAC were significantly lower in both the ASD subgroup with language delay and the very small subgroup ($n=5$) without language delay compared to controls, and the two ASD subgroups did not significantly differ from each other; Cho/Cr was higher in the left HAC in the language-delayed ASD subgroup compared to both the non-language-delayed ASD group and typical controls; mI/Cr was higher in the left and right HAC in the language-delayed ASD subgroup, but not in the non-delayed group, compared to typical controls.

In the cerebellum: there were no NAA/Cr subgroup differences; Cho/Cr was higher in the language-delayed ASD subgroup, but not in the ASD non-delayed language subgroup, compared to typical controls; mI/Cr was higher in both language-delayed and non-language-delayed ASD subgroups than typical controls. Larger samples are needed to determine if the findings can be replicated and expanded.

9.3.14 What Do MRS/MRSI Results Suggest About Neurodevelopmental Mechanisms in Autism?

As with other types of neuroimaging studies of autism, there are many sources of biological and nonbiological variation in MRS/MRSI studies. The studies with the largest samples of carefully characterized and matched ASD and comparison participants, and those performed under standardized conditions with the most rigorous methodology for image acquisition, processing, and analysis and the highest standards of quality control, will decrease nonbiological variation and increase power to find true biological differences of interest. In this review, we assessed and tried to integrate and summarize results across the many MRS/MRSI studies that have been done in autism research. In the process of doing so, we were inevitably dealing with tremendous nonbiological variation due to less than optimal methodology in some of the studies, lack of standardized methods across studies, and the inherent challenges in MRS/MRSI, as well as the inherent marked clinical and biological heterogeneity of ASD. In particular, only a few studies had sufficient sample sizes to approach the ability to distinguish 10 % differences in NAA with 80 % power. Since all other measured metabolites have smaller peaks and as a result greater error, the power to detect differences in these smaller signal peaks is markedly reduced. Bearing in mind the fact that studies on small samples are more likely to yield anomalous findings, contradictory results are to be expected, particularly for the lesser metabolites. As a result, the majority of observations we made can at most only be considered trends and tendencies that need to be replicated in much larger state-of-the-art studies before any firm conclusions can be made.

The results from the studies in very young children with ASD provide the most helpful information about mechanisms proposed to be involved in autism. First, the MRSI study in 3–4-year-old children provides strong evidence for a specific abnormality of gray matter early in the clinical course of autism (Friedman et al. 2006). Case–control findings in gray matter were more specific to autism than results in white matter (Table 9.4). The white matter metabolite differences were more nonspecific, albeit still abnormal; they could represent pathology shared by ASD and non-autistic developmental delay. The investigators state that the gray matter findings could be the result of differences in gray matter connectivity or density. Second, the investigators hypothesized, because of the tendency toward brain overgrowth in early childhood in autism, that NAA would be increased (Friedman et al. 2003). Instead, NAA was decreased. This is not necessarily incompatible with more neurons in the larger autism brains, but it does imply that either the neuronal density in the larger ASD brains is decreased or that there is some impairment of neuronal function or connectivity. Third, multiple metabolite findings in the young ASD children were not compatible with the hypothesis of frequent mitochondrial dysfunction in the disorder. Neither mean Lac^- nor the rate of Lac^- greater than 2 standard deviations above the control mean was increased in the ASD sample, at 3–4 years of age or at follow-up examinations when the children were 6–7 and 9–10 years old (Corrigan et al. 2012). Glx concentrations were not increased in gray matter or

white matter in the young children with ASD. Of the 6 other studies of ASD that have examined Glx or glutamate by itself in older children and adults, all studies either found no differences or a decrease in concentration (DeVito et al. 2007; Bernardi et al. 2011; Corrigan et al. 2011; Harada et al. 2011; Kubas et al. 2012), except for the study in the very oldest individuals (mean age 35.6 years), which found increased glutamate in the right hippocampal–amygdala complex (Page et al. 2006).

9.4 Conclusion

As is hopefully evident from the information provided in this chapter, MRS studies of neurochemistry in individuals with an ASD have contributed some tantalizing hints about understanding of the neurobiology of autism and will continue to do so in the future. There are several important factors that should be kept in mind for future MRS studies of autism. First, there is a great need for longitudinal MRS studies to observe how the concentration of various neurochemicals changes throughout development and across the life span in individuals with an ASD, and to compare those findings to those in both TD and other DD samples. Such studies are already underway, as previously discussed, and it will be very interesting to learn the results as they are reported in the literature and to compare those findings with the numerous cross-sectional MRS studies of autism already completed.

Another factor that will greatly increase the reliability of study results is to ensure that MRS methodology is both optimized and consistent across studies. MR system quality assurance (SQA) and quality control of all spectral data prior to analysis is vital to have confidence in comparisons between studies from different groups (Kreis 2004; van der Graaf et al. 2008). Performing adequate corrections for partial volume effects during data analysis is essential for valid estimates of metabolites in larger volumes of gray and white matter. Assessing smaller structures like the amygdala and hippocampus requires creative approaches to eliminating contamination from adjacent brain structures that may have very different metabolic profiles (Nacewicz et al. 2012). Ideally, investigators will agree on one or two standard MRS methods, with uniform pulse sequence parameters and standard analysis procedures, when examining the same major regions of interest across studies. Doing these things would not only increase confidence in reported results but would also make it much more valid to compare results across studies to develop a big picture of neurochemistry in autism.

A third such factor is that potential confounds should be considered in all MRS studies of autism, the heterogeneity of which is already a significant source of variability in the data. Such potential confounds include but are not limited to the effects on neurochemistry of sedation, medication use, type of diagnosis and severity of symptoms, sex (male vs. female), level of cognitive function, and age. A final factor is that sample sizes of both autism and comparison groups in MRS studies of autism need to be greatly increased in future studies in order to have sufficient power to detect differences, particularly important given the inherent insensitivity of MRS. To summarize, high-quality large-sample studies are needed for future MRS autism research.

We will end this chapter with a look at where studies of neurochemistry in autism are heading. One novel method, diffusion tensor spectroscopy (DTS), which combines MRS and diffusion tensor imaging (DTI) technology, has the potential to open a whole new aspect of spectroscopy research in autism. Traditional DTI uses the diffusion properties of water to measure microstructural characteristics of cerebral white matter fiber tracts. Because water in the brain is located both within cells (intracellularly) and outside of cells (extracellularly), it is often not possible to determine whether changes in diffusion tensor coefficients, which describe properties of brain microstructure, are due to abnormalities within or outside of cells, and if in cells, then what cell type (e.g., neurons or glia) and what part of the cell (e.g., inside the cell body of a neuron or in the myelin sheath around the axon). DTS measures the diffusion of brain metabolites instead of water. In practice, only metabolites with the strongest MRS signals can be studied with DTS. To date, DTS studies have only looked at *N*-acetylaspartate (NAA), which is mainly present in neurons, including their axons. Measuring NAA diffusion properties with DTS and comparing NAA diffusion to that of water provide tissue-specific information about changes in brain microstructure that involve neurons and their axons. Although there are no published studies of diffusion tensor spectroscopy in ASD subjects, DTS is being studied in other brain disorders (Wood et al. 2012) and holds promise for detecting abnormalities in brain microanatomy that may be relevant to the primary pathology of autism (Casanova et al. 2002; Casanova 2006; Casanova and Trippe 2009). Using DTS to study autism will allow researchers not only to compare neurochemistry between ASD and other groups but also to track the diffusion of particular neurochemicals into and throughout various brain regions.

One possible future course for MRS studies of autism is the investigation of the combination of neuroimaging and genetics. This is likely to play an important role in future studies of the neurochemistry of autism, as findings from neuroimaging studies can be used to inform genetics studies and vice versa. The Endo et al. (2010) study is an excellent example of prior findings in genetics being used to devise a study of neurochemistry in autism, by looking at how neurochemistry (NAA/Cr and Cho/Cr in this case) might differ in certain brain regions when comparing ASD individuals with either the S/S or S/L genotype of the *5-HTTLPR* polymorphism. Finding differences in neurochemistry between ASD samples and control samples could inform researchers about potential genetic causes, such as abnormalities in neurochemical transporter genes. The best possible outcome of future MRS autism research is that neurochemical biomarkers will be found that can classify an individual as having an ASD phenotype, perhaps based on neurochemical concentrations in certain brain regions. This possibility alone makes MRS an important avenue for future autism research.

There have also been major technical advances in NIR spectroscopy in the past decade. Of great interest in regard to potential future applications is the development of lightweight wireless systems that can be worn by infants and young children, not only the research lab but also in ambulatory settings (Elwell and Cooper 2011). At present, these wireless systems are only CW (continuous-wave) devices, and this poses considerable obstacles to the kind of quantitation required for intra-subject and intra-group comparisons. However, there are potential work-arounds for the quantitation

problem (Hoshi 2003), and technological innovation is moving rapidly in NIRS. The wireless NIRS devices already permit real-time measurements of regional cerebral blood flow (rCBF) during task activation. The other application of NIRS, optical imaging of rCBF and the oxygenation state of mitochondria (from cytochrome oxidase), is also an area of intensive research. NIR optical imaging can assess brain functions at the bedside and measure rapid regional changes during cognitive tasks. NIR optical imaging is thus a technique that enables novel neuroimaging studies that provide information complementary to that provided by MRS and MRI (Table 9.14).

Table 9.14 Glossary of imaging terms

MRI/MRS terminology	Description/definition	References
Absolute quantitation	A reasonably accurate method for calculating metabolite concentrations from the area under MRS spectral peaks. For MRS, all concentrations computed are computed as a ratio to some reference signal (Alger 2010). The term "absolute quantitation" is often used to denote concentrations calculated taking into account the appropriate relaxation factors for both the metabolite and whatever the reference signal is and also the volume from which the metabolite signal comes. The reference signal may be from a source outside the brain ("external reference") or from a source within the brain—commonly either brain water resonance or the main Cr peak at 3.05 ppm ("internal reference"). More commonly, the so-called concentrations are not corrected for relaxation effects, in which case they can only be compared to the same metabolite in other studies that used the same pulse sequence, TR and TE	Alger (2010)
Filtering	A mathematical operation that "smoothes" the spectrum to reduce the noise. Filtering always increases the width of spectral lines somewhat	
LC model	The most popular standard method for automatic analysis of 1H MR spectra to determine peak positions and the areas under those peaks. It uses nonlinear least-squares optimization algorithms to fit the spectrum simultaneously to a "basis" set of metabolite peak positions and widths. The basis set is MR spectra acquired from a **phantom** containing a solution of metabolites of known concentration, using the same pulse sequence and data processing that are used in the human MRS exams. Such automatic spectral fitting is a prerequisite for determining the relative or "absolute" concentrations of metabolites from MR spectra. It is, however, fraught with difficulties—the extended computation time for large numbers of spectra (such as MRSI produces)—which can be 8 h or more per subject and uncertainty about the validity of fitted values	Provencher (1993); Alger (2010)

(continued)

Table 9.14 (continued)

MRI/MRS terminology	Description/definition	References
Magnetic resonance spectroscopy (MRS)	**M**agnetic **r**esonance **s**pectroscopy (MRS) uses the MRI scanner to obtain spectra that let us determine the concentrations of brain metabolites and thus the dynamic metabolic activity within the brain	
Magnetic resonance spectroscopic imaging (MRSI)	**M**agnetic **r**esonance **s**pectroscopic **i**maging is an MRS method that acquires MR spectra from many voxels simultaneously, in one or several planes across the brain. Other names for MRSI are spectroscopic imaging and chemical shift imaging	
Partial-volume analysis and correction in MRS	In MRS, because of its very large voxel sizes, the voxels rarely represent pure GM or pure WM. The voxel might have some combination of GM, WM, and/or CSF. Partial volume analysis estimates the proportion of GM, WM, and CSF in each voxel, to give a more accurate estimate of global brain volume, for example, or to allow more accurate calculation of metabolite concentrations	
Phantom	In MRI/MRS, a test object, for example, a bowling-ball-sized sphere containing a solution of specified composition that contains water with a specific T1 or a solution of metabolites	
PEPSI	**P**roton **e**cho-**p**lanar **s**pectroscopic **i**maging: an extremely rapid method of spectroscopic imaging. Its disadvantage is its sensitivity to distortions in MRS frequencies produced by bone-air or bone-tissue interfaces (such as the frontal sinus)	Nacewicz et al. (2006); Page et al. (2006)
Shimming	The optimization of the main static magnetic field, B0, such that it becomes largely independent of the spatial coordinates over the region of interest. This minimizes the difference between T2 and T2* and makes the position (frequency) of spectral peaks largely independent of the spatial coordinates for MR spectroscopy	Gruetter and Boesch (1992)
Single-voxel MRS (SV MRS)	Methods that acquire a spectrum from a single volume (voxel). The most common pulse sequences are PRESS (point-resolved spectroscopy) and STEAM (stimulated-echo acquisition spectroscopy)	Bottomley (1989); Frahm et al. (1989)
Spectral editing technique	Spectral editing sequences are advanced methods of acquiring MR spectra that allow measurement of metabolites that are impossible to measure accurately with standard MRS, because of overlapping peaks or because they are multiplets. Spectral editing can measure the amounts of GABA, glutamate, or glutamine more accurately. Some editing techniques require separate editing acquisitions for each metabolite, whereas others, such as 2D J-resolved ^{1}H MRS, can measure multiple metabolites simultaneously	

(continued)

Table 9.14 (continued)

MRI/MRS terminology	Description/definition	References
T2*	T2* (T-two-star) is the native T2 of the water protons plus the effect of magnetic field gradients (caused by tissue differences or artifacts of the pulse sequence), which also cause the spins to dephase. T2* is always shorter than T2	
Tesla	The strength of an MRI scanner's main magnetic field (B) is expressed in Tesla (T). The most common field strengths for clinical MRI are 1.5 T and 3.0 T, although there are research scanners with stronger (higher) fields	
Voxel	A three-dimensional volume element of an MR image: "Voxel" in the three-dimensional analogue of "pixel" which is an element of a two-dimensional picture or image	
Zero filling	A mathematical operation that results in doubling of the number of voxels in the dimension that is zero filled	

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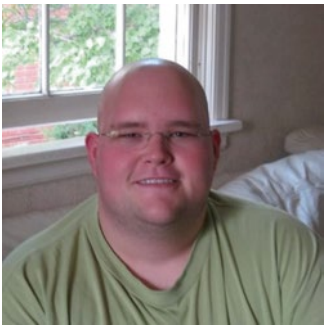

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Chapter 10
Functional MRI in Autism

Jeffrey S. Anderson, Michael A. Ferguson, and Jared A. Nielsen

10.1 Introduction

Functional MRI is a technique that measures changes in blood oxygenation associated with neural activity to make inferences about which regions of the brain are more "active" during a cognitive task than during a comparison task. It has been found that the blood flow measurements obtained by blood oxygen level-dependent (BOLD) images are tightly coupled to underlying neural activity with a spatial scale of millimeters and a temporal scale of seconds (Logothetis 2002). Even brief neural activations, as measured using light-activated optogenetic techniques, can successfully trigger spatially localized signal increases in the region of stimulation (Lee et al. 2010).

Since the first application of functional MRI to study autism populations in 1999 (Baron-Cohen et al. 1999; Ring et al. 1999), there have been nearly 200 reports or meta-analyses of functional MRI (fMRI) or functional connectivity MRI (fcMRI) data in autism. Many excellent review articles have been published highlighting convergent features of these studies, some combining information from functional and structural imaging (Cody et al. 2002; Williams and Minshew 2007; Verhoeven et al. 2010; Pina-Camacho et al. 2011; Stigler et al. 2011) and others reviewing

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primarily functional MRI studies of autism (Minshew and Keller 2010; Philip et al. 2012) or functional connectivity studies (Kana et al. 2011a; Muller et al. 2011; Schipul et al. 2011; Vissers et al. 2012), which are discussed in a separate chapter.

In this chapter, a broad selection of task-related fMRI studies are reviewed to determine what changes during cognitive tasks can be observed in autism, what unifying principles might underlie such changes, and how can these techniques be used to guide or constrain diagnostic and therapeutic decisions regarding autism. Ultimately, published reports of abnormalities in fMRI data in autism show heterogeneity of involved brain networks, with most studies limited by relatively small sample sizes. Nevertheless, trends toward hypoactivation in complex, distributed brain networks emerge, with some networks affected more than the others. fMRI reports are grouped below by neural subsystem. It is noted that many reports might be classified in multiple sections and the studies reviewed below have been grouped to facilitate a discussion of abnormalities in different neural subsystems rather than provide a comprehensive review of any one neural network.

10.2 Auditory Processing

Early studies of auditory processing in autism using functional imaging techniques were performed with single-photon emission computerized tomography (SPECT) (Garreau et al. 1994) and positron emission tomography (PET) (Muller et al. 1999) and found hemispheric asymmetries during stimulation with auditory tones. Both studies found greater activation in the right hemisphere in response to stimulation in autism, with relatively greater activation in the left hemisphere in control subjects. A PET study by Boddaert and colleagues performed during auditory tone stimulation also found a greater volume of activation in autism in the right hemisphere (Boddaert et al. 2003), with control subjects showing greater activation in the left temporal lobe.

Using a paradigm with both vocal and nonvocal sounds, Gervais and colleagues used fMRI to find that autism subjects showed relatively less activation of the bilateral superior temporal sulcus for vocal sounds, but normal activation for nonvocal sounds (Gervais et al. 2004). Nevertheless, sample sizes were small (five autism and eight control subjects). Subsequent studies have been reviewed by O'Connor (2012) and include primarily studies involving language stimuli, which are discussed below. One additional study measuring phonological processing in parents of individuals with autism (Wilson et al. 2012) found greater suppression of activity for stimuli associated with phonological priming in areas relevant to early language processing such as superior temporal and supramarginal gyrus.

Thus, even at very early (non-language or pre-word) auditory processing stages, functional imaging data suggest hemispheric asymmetries and reduced activation of temporal auditory processing regions. These results are broadly consistent with magnetoencephalography (MEG) data showing delayed responses to simple tones within the right hemisphere (Roberts et al. 2010) and decreased gamma power in steady-state responses to repeated click stimuli in the left hemisphere (Wilson et al. 2007).

The implication of these results is that even in primary sensory processing, delays and reduced brain activation are seen that may propagate through later stages of auditory and language processing to generate the behavioral deficits in language seen in children and adults with autism.

10.3 Language

A number of studies show decreased activation of left hemispheric language regions during an assortment of language tasks. During passive listening to prerecorded speech, Lai and colleagues found decreased brain activation in autism in bilateral superior temporal gyrus (Lai et al. 2011). Decreased activity in Broca's area (but increased activity in Wernicke's area) was reported during a semantic processing task (Harris et al. 2006) and during a sentence comprehension task (Just et al. 2004). Reduced activation in Broca's area was also observed in a word categorization task (Gaffrey et al. 2007) (Fig. 10.1).

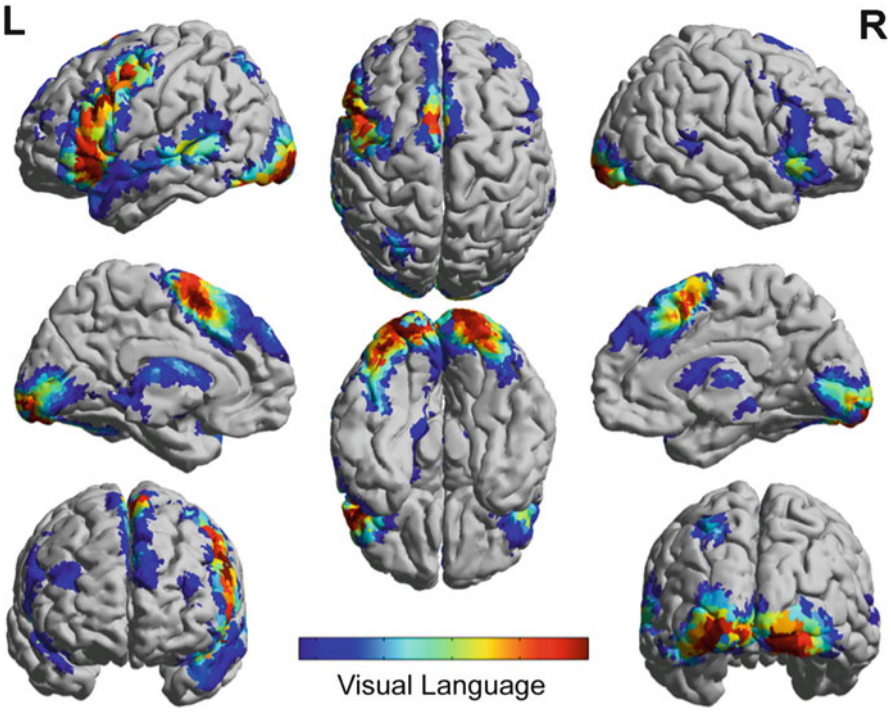

Fig. 10.1 Brain regions activated in 25 typically developing subjects during a reading task. Subjects were required to read a sentence and fill in a missing word at the end that would complete the sentence. Activated areas include visual cortex, Wernicke's area, Broca's area, superior temporal sulcus, supplementary motor area, and dorsolateral prefrontal, and lateral premotor cortical regions

In tasks requiring more complex language function or inferences about the speaker that require knowledge of the speaker's gender, social class, or age, individuals with autism showed reduced activity of the left frontoinsular region (Tesink et al. 2011) and reduced left inferior frontal activity for social contrasts such as incongruity of speaker's age or social characteristics (Groen et al. 2010). A task that required processing nonsense syllables with the prosody of an artificial language demonstrated reduced activation in both Broca's and Wernicke's area in autism relative to controls (Scott-Van Zeeland et al. 2010b).

Similarly, more complex language processing regions, such as the insula (Uddin and Menon 2009; Anderson et al. 2010) and superior temporal sulcus (Redcay 2008), appear most likely to show subtle differences in activation in autism and correspond to deficits in intonation, social inferences, and prosody that are most evident in high-functioning autism.

Nevertheless, differences are small with large intersubject variability that is greater than the effect size of autism, and several studies report increased activation in language regions in autistic subjects. Increased activation in left supramarginal gyrus was reported by Hesling et al. (2010), although also noting reduced deactivation of default mode regions associated with processing of internal stimuli or internal narrative during language, as has been observed in other tasks (Kennedy et al. 2006). Increased activation in left Broca's area was also reported by Knaus and colleagues (2008).

Individuals with autism typically show less impairment for music than for language, and studies evaluating musical stimulation have shown accordingly fewer abnormalities in autism. In a study measuring both speech and song, autism subjects showed decreased supramarginal gyrus activation for speech, but not for song (Lai et al. 2012). Caria and colleagues found during stimulation with happy and sad musical clips that autism subjects showed decreased activation of the left anterior insula, particularly during happy passages (Caria et al. 2011).

Several studies report abnormal language lateralization with either increased right-hemispheric recruitment during language or decreased left lateralization (Takeuchi et al. 2004; Kleinhans et al. 2008a; Knaus et al. 2008; Tesink et al. 2009; Anderson et al. 2010) apparent as early as 2 years of age (Redcay and Courchesne 2008; Eyler et al. 2012).

Accessory language areas are reported in several studies where autism subjects show atypical activation of parietal or occipital lobe regions. For example, a task that required processing of mental imagery during language showed activation of parietal lobe regions more frequently for autism subjects (Kana et al. 2006). A study that used movies as stimuli showed atypical activation patterns in autism subjects that were more variable than in control subjects (Hasson et al. 2009).

From a review of the figures of brain activation embedded in these studies, the network of brain regions subserving language function in autism is organized with precisely the same architecture as in typically developing individuals, with similar levels of activity in comparable brain regions that are distinguished only by

statistical and not qualitative methods. Nevertheless, several trends are reproduced across multiple studies:

- Core left hemispheric language regions show diminished fMRI activation in autism. This is especially true of speech production areas (Broca's area is more often abnormal than Wernicke's area), complex auditory processing areas (superior temporal sulcus and insula are more often affected than primary auditory cortex), and tasks requiring complex social inferences about the speaker or more abstract patterns of language such as artificial languages.
- Individuals with autism show decreased left lateralization of language compared to typically developing controls, with greater recruitment of right-hemispheric homologous regions. Language lateralization is abnormal as early as 24 months of age.
- Accessory language areas including parietal and occipital lobe are more commonly seen with autism or recruited at lower semantic complexity thresholds.
- Activation differences are more pronounced for speech than for music, possibly because music already recruits right-hemispheric language areas in controls, making differences less pronounced.
- There is reduced deactivation of the default mode network during language processing in autism.

These findings are consistent with results from functional connectivity studies in autism showing reduced interregional synchrony among brain networks, with deficits becoming more severe for tasks requiring integration of distributed information through successive layers of neural processing. Whereas only slight delays are seen in basic auditory processing, generally seen only in electrophysiological measurements at fine temporal resolution, deficits become more apparent for complex language or social language processing tasks, with the autistic brain first recruiting accessory language regions in the right hemisphere and parietal lobes, and ultimately failing to deactivate networks that process internal stimuli or narrative during language.

10.4 Touch Perception

A small, recent literature has explored the neural substrate for atypical touch perception in autism. One paradigm for evaluating touch is the rubber hand illusion, where synchronous tactile and visual stimulation result in the illusion of "owning" an artificial hand. In one study, autistic children were less susceptible to this illusion, requiring 6 min of stimulation to generate the illusion (Cascio et al. 2012a). An observational study that also included parent reports of tactile stimuli hypo- or hyperresponsiveness found that hyporesponsiveness was strongly correlated with social and communication impairments of autism and sensory seeking was associated with social impairment and repetitive behaviors (Foss-Feig et al. 2012).

An fMRI study used a paradigm where participants felt three textures that ranged from pleasant to abrasive. Behavioral responses to the textures showed that autism participants tended to rate both the pleasant and unpleasant stimuli as more extreme (Cascio et al. 2012b). For pleasant and neutral stimuli, autism participants showed decreased activation of somatosensory cortex, but for the unpleasant texture, autism subjects showed greater activation of posterior cingulate and insula, areas associated with affective responses to stimuli (Cascio et al. 2012b).

10.5 Visual Feature Perception

Autism subjects show relatively minor, if any, impairments in visual feature processing. A study evaluating the retinotopic organization of primary visual cortex detected no abnormalities in organization or activity levels (Hadjikhani et al. 2004a). Moreover, behavioral results typically show that tasks involving visual search or the embedded figures task, where subjects must identify a subfigure within a larger figure, are often performed at normal levels. Several studies have investigated visual feature perception during acquisition of fMRI images.

Embedded figure tasks have been performed in at least four studies (Ring et al. 1999; Lee et al. 2007; Manjaly et al. 2007; Malisza et al. 2011). In all four studies, autism participants exhibited normal or increased activation of visual cortex and lateral occipital regions and relatively normal activation of visual attentional regions along the medial intraparietal sulcus. Yet in all four studies, there was decreased activation in the frontal lobes in autism, including anterior cingulate (three studies) and lateral prefrontal and premotor cortex (three studies). Attentional regions in the supplementary motor area, frontal eye fields, area MT, and bilateral caudate nuclei also showed decreased activation across multiple studies.

A task involving mental rotation similarly demonstrated decreased activation in autism within the frontal eye fields, anterior cingulate, and caudate nuclei (Silk et al. 2006). In a study where participants were required to count colored edges in 3D figures, where the 3D image may serve as a distractor, autism subjects demonstrated decreased activation of dorsolateral prefrontal cortex (Liu et al. 2011). Only one study found conflicting results using a visual search task that showed increased frontoparietal activation in autism (Keehn et al. 2008). A meta-analysis of published literature using an activation likelihood estimator confirmed that autism subjects across many studies evaluating objects, faces, and word stimuli showed increased activation in occipital, temporal, and parietal regions but decreased involvement of frontal lobe attentional regions during task performance (Samson et al. 2012).

10.6 Motion Perception

A number of studies have now investigated brain activation in autism related to motion perception. In this paradigm, typically a pattern of dot stimuli is manipulated to show either random or coherent motion versus biological motion.

The typical brain activation pattern for motion perception involves earliest detection of motion in area V5/MT, with higher-order visual attentional areas along the medial intraparietal sulcus and in the superior temporal sulcus also showing greater activation for biological than for coherent or randomly moving dot patterns.

In autism, studies have generally shown decreased activation in inferior parietal lobule, frontal eye fields, and superior temporal sulcus (Herrington et al. 2007; Freitag et al. 2008; Brieber et al. 2010; Kaiser et al. 2010; Koldewyn et al. 2011) with variable activation within area V5/MT and primary visual cortex (Brieber et al. 2010). A study of 62 subjects including unaffected siblings demonstrated that both siblings and autism participants showed decreased activation within V5/MT or superior temporal sulcus, with possible compensatory increased activation in siblings in scattered medial prefrontal, posterior temporal, and inferior parietal regions (Kaiser et al. 2010).

These results are broadly consistent with studies on visual feature perception where abnormalities in autism consist of hypoactivation of downstream frontal and temporal attentional regions with variable abnormality involving earliest brain regions processing the features of interest.

10.7 Motor Function

Both fine and gross motor skills are typically impaired in autism (Fournier et al. 2010). An early evaluation of a simple finger movement task in autism showed that the organization of brain activation involving motor cortex, basal ganglia, thalamus, supplementary motor area, and cerebellum was similar for autism and control subjects. Yet, activity in the motor and supplementary motor cortices was decreased in autism, with reduced deactivation of default mode network regions (Muller et al. 2001) (Fig. 10.2).

In a series of tasks designed to measure cerebellar performance where subjects either pressed a button continuously (motor) or pressed a button in response to cues (attentional), autism subjects showed decreased cerebellar motor activation only for the attentional task but increased cerebellar activation for the pure motor task (Allen and Courchesne 2003). A follow-up study confirmed these findings and found that hyperactivation in the motor task was correlated with structural volumetric abnormalities in the cerebellum (Allen et al. 2004). A contrasting result was obtained in children by Mostofsky et al. in which subjects performed appositional finger tapping, with autism subjects showing reduced activation in the ipsilateral cerebellum but greater activation in the supplementary motor area (Mostofsky et al. 2009).

10.8 Oculomotor Control and Gaze Perception

Abnormalities have been observed in autism involving control of gaze and abnormal eye contact in social interactions (Itier and Batty 2009). To study this effect using fMRI, Pelphrey and colleagues used cartoon faces that deviated their eyes either toward (congruent) or away (incongruent) from approaching stimuli. Whereas

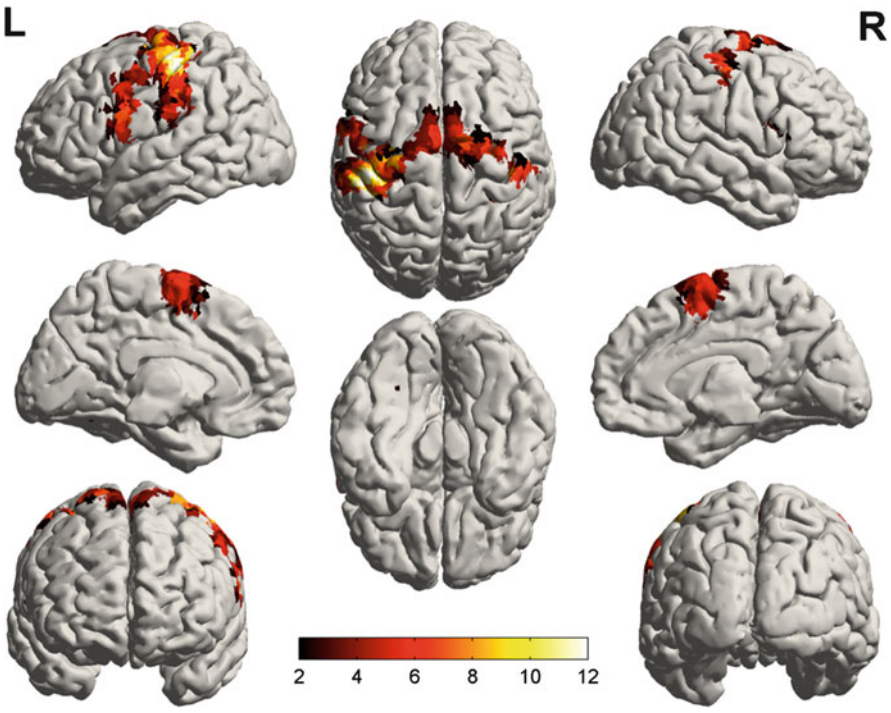

Fig. 10.2 Brain areas activated when 15 typically developing subjects moved their right fingers. Activated areas include primary motor cortex, particularly in the left hemisphere, supplementary motor area, and left premotor cortex

control subjects showed significantly increased activation in inferior parietal lobule and superior temporal sulcus regions for incongruent trials, autism subjects showed weaker activation in both regions with little difference between congruent and incongruent trials (Pelphrey et al. 2005). In a study evaluating an oncoming male figure with either direct or averted gaze, only control subjects showed increased activation in the right insula and right temporoparietal junction in association with direct gaze compared to averted gaze (Pitskel et al. 2011).

In a task where participants were shown emotional faces with either direct or averted gaze, autism children showed reduced activation in ventrolateral prefrontal cortex compared to controls, with no difference between direct and averted gaze stimuli (Davies et al. 2011).

A study requiring participants to make a response as directed by a target word ("left" or "right") in the presence of distracting information included both arrows and a face with deviated gaze to one side (Vaidya et al. 2011). Greater interference that was seen for autism children associated with arrow stimuli was higher than for control children. In another study where subjects viewed a panel of faces either looking in the same direction or variable directions, autism subjects showed activation in the superior temporal sulcus, temporoparietal junction, intraparietal sulcus, and

amygdala to coherent compared to variable gaze direction that was inversely related to disease severity as measured by autism quotient (Nummenmaa et al. 2012).

When subjects were required to make either saccadic or smooth pursuit eye movements, this resulted in decreased activation in frontal eye fields and cerebellar hemispheres for both tasks (Takarae et al. 2007). An increase in activation in dorsolateral prefrontal cortex, caudate, thalamus, and cingulate cortex was seen during visually guided saccades, interpreted by the investigators as increased task difficulty requiring recruitment of additional brain regions (Takarae et al. 2007).

In a study examining response inhibition involving saccadic eye movements, autism participants showed reduced activation of both frontal eye fields and anterior cingulate cortex in response to antisaccades compared to the less difficult prosaccades (Agam et al. 2010). A subsequent magnetoencephalographic (MEG) study examined the short-time interval prior to a saccadic eye movement and found reduced coherence between bilateral frontal eye field and anterior cingulate cortex in autism (Kenet et al. 2012). In another study evaluating saccade and antisaccadic control, autism subjects showed increased activation in the anterior cingulate for correct trials that may be interpreted as a misleading signal prompting correction. Nevertheless, autism subjects showed reduced fractional anisotropy in a diffusion tensor analysis in the underlying cingulate white matter (Thakkar et al. 2008).

Across studies, the trend again favors decreased activation in autism in higher-order oculomotor processing regions for saccade, gaze perception, and gaze-related response inhibition tasks, consistently varying with disease severity and exhibiting decreased functional interregional synchronization between relevant brain regions.

10.9 Perception of Faces

Neural processes involved in facial perception differ between neurotypical and autistic populations (Weigelt et al. 2012). It may be inferred that processing facial stimuli requires greater visuospatial effort for autistic individuals than for neurotypical persons (Hubl et al. 2003). The increased difficulty in facial processing for autistic individuals relative to control subjects can be attributed to the inherent social content of face stimuli, as opposed to an increase in the sophistication of visual demand itself (Bookheimer et al. 2008).

In typically developing individuals, a number of brain regions are preferentially recruited in response to emotionally expressive faces versus emotionally neutral faces. These regions include the fusiform face area, extrastriate cortices, mesolimbic regions, amygdalae, and temporal lobes. Repeatedly, experiments have demonstrated reduced responses in combinations of these areas in autistic individuals when presented with emotionally expressive faces, compared to activity levels in typically developing individuals for corresponding tasks (Critchley et al. 2000; Deeley et al. 2007). Hypoactivation of amygdalae and fusiform face areas, in particular, has been highly reported for autistic individuals processing emotionally expressive facial cues (Critchley et al. 2000; Dalton et al. 2005; Deeley et al. 2007; Ishitobi et al. 2011).

A possible explanation for hypoactivation of fusiform and amygdaloid regions in autistic individuals during facial perception might be the effect of gaze on stimulating these neural areas. In both control and autistic participants, activation of the fusiform gyrus and the amygdalae was strongly, positively correlated with the duration of time that a participant fixed their gaze on a face. As such, it is reasonable that reduced fusiform activity following the presentation of face stimuli to autistic subjects may be the result of aberrant gaze fixation behavior, rather than fusiform dysfunction (Hadjikhani et al. 2004b; Dalton et al. 2005).

Observation of hypoactivity in amygdalae and fusiform areas has not been unanimously supported in research findings. Presentation of anxious faces by Hall et al. did not affect the time course or intensity of amygdala activity in autistic subjects compared to neurotypical controls. The fusiform face area, however, did manifest decreased activity comparing autistic subjects to controls (Hall et al. 2010).

Contradictory observations of amygdaloid responsiveness in autistic versus control groups might be reconciled by carefully accounting for gaze fixation time. When fixation time is controlled, autistic individuals were shown to have an *increase* in amygdaloid activation in response to facial photographs, compared to neurotypical control subjects. Avoidance of gaze fixation on face stimuli, generally seen in autistic individuals, may be a protective mechanism to avoid neural overresponsiveness to facial stimuli. Consequently, reduced activity of fusiform areas in autistic individuals might be secondary to averted gaze, motivated by avoidance of amygdalae overstimulation (Dalton et al. 2005).

Functional connectivity analysis corroborates biological contributions to differential amygdala and fusiform behavior between autistic and neurotypical individuals, with greater degrees of social impairment corresponding to decreases in functional correlations between fusiform face areas (Kleinhans et al. 2008b). In other words, the observations of aberrant amygdaloid and fusiform responses in autistic individuals are likely to be multifactorial.

There is mounting evidence that the amygdalae in autistic persons do function differently than the amygdalae in neurotypically developing individuals. For example, neurotypical study participants manifested bilateral amygdalae activation in response to the presentation of whole faces, but not to visual presentation of parts of faces. In contrast, autistic participants were particularly responsive to amygdala stimulation by visual presentation of lower facial parts, especially the mouth (Ishitobi et al. 2011).

In addition to potential contributions of amygdaloid and fusiform regions in differential processing of faces between autistic and control subjects, speculation exists that mirror neuron disturbances may also be complicit. Face presentations elicit weakened inferior frontal cortex and superior temporal sulcus activity in autistic individuals compared to typically developing controls (Hadjikhani et al. 2007). Inferior frontal cortical activation was also observed in a study where subjects viewed partial faces and made “self” or “other” judgments (Uddin et al. 2008).

10.9.1 Social and Empathic Reasoning

The identification of a "social brain," or a network of neural subregions specifically dedicated to processing social information, includes the orbitofrontal cortex, superior temporal gyrus, fusiform face area, amygdala, and mirror neuron systems. Though not constrained to investigation of these subregions, these areas have naturally become prime suspects of investigation when trying to understand differences in neural functioning associated with social behavioral differences observed in autism spectrum disorders.

Early findings demonstrated that, indeed, typically developing individuals recruit superior temporal and amygdaloid areas for cognitive tasks related to inferences about another person's emotional state. Although frontotemporal areas were also activated for autistic subjects performing a theory of mind task, there was significant reduction of amygdalar recruitment, indicating that key task content is either not being processed or is being processed differently between neurotypical and autistic participants (Baron-Cohen et al. 1999). In conjunction with behavior measures showing less accurate performance on tasks for discerning social intent, increased recruitment of frontal and temporal regions in these tasks indicates that discernment of intent may be a more cognitively demanding process for persons with autism, independent of the sensory modality being communicated (Wang et al. 2006).

The increase in task difficulty for socially related paradigms in autistic individuals compared to typically developing persons is likely related to disruptions in normal recruitment of attentional networks when social stimuli are presented. More generally, cognitive control during states of arousal is impaired in autism compared to typically developing brains (Dichter and Belger 2007). Even when behavioral measures for social tasks are not robust enough to detect group differences between autism and neurotypical subjects, functional imaging outcomes may still elucidate variations in neural responses to nonsocial versus social stimuli in autism subjects contrasted with neural events in typically developing subjects. In conjunction with observations of attentional modulation during social stimuli, a meta-analysis of 39 studies revealed that the right anterior insula is more likely to be hypoactive in autistic individuals during social paradigms, compared to neural performance in nonsocial paradigms (Di Martino et al. 2009). For example, multiple studies on social exclusion have shown that simulation of peer exclusion in the laboratory setting results in hypoactivation of the right anterior insula, in contrast to nonsocial rule violations resulting in hyper-recruitment of the right anterior insula (Masten et al. 2011). The emergent viewpoint is that autistic brains do not assign social cues the same privileged status that they receive in typically developing systems and that normative behavioral outcomes in experimental contexts may represent learned compensatory mechanisms at the neural level, rather than indicating typically functioning social attention (Greene et al. 2011).

In addition to social stimulation of attention control, the mirror neuron system is an integral part of normal social cognition and behavior. In typically developing brains, mirror neuron activity is partially reflected by somatosensory activity during

theory of mind and emotional recognition tasks. In both theory of mind and emotional recognition experiments, there is substantial decrease in somatosensory activity for autistic individuals compared to neurotypical controls (Sugranyes et al. 2011). Mirror neuron activity, however, appears to increase in autistic individuals as they age as evidenced by increasing activity in the inferior frontal gyrus in the execution of socially relevant tasks, with accompanying improvement in social function (Bastiaansen et al. 2011).

On the whole, the most promising explanatory models for social behavioral deficits integrate mechanisms across multiple neurobiological systems. Though not explicitly evidenced from fMRI, it should be noted that significant progress in elucidating the underlying neurochemistry in autistic social deficits has made compelling strides forward in relating dopamine and oxytocin pathways to the overall schema of socially relevant behavior and cognition. Naturally, more complete understanding will emerge from integrated models of neurochemistry and functional anatomy (Neuhaus et al. 2010).

10.10 Emotional Processing

Recognition and interpretation of emotional states plays key significance in autism. One major vehicle for conveying emotionality is through body gesturing and expression. In neurotypically developing individuals, multiple brain regions are preferentially activated when another person is observed to express bodily gestures associated with fear (compared to observing an emotionally neutral body pattern). Between autistic and neurotypically developing persons, the anterior insula and inferior frontal cortex are significantly less aroused by the observation of fearful versus neutral gesturing (Hadjikhani et al. 2009). Other studies have similarly reported hypoactivation of amygdalae, inferior frontal, and premotor cortices when autistic individuals observe fear gestures, compared to typically developing individuals observing the same stimuli (Grezes et al. 2009). Reasonable questions may be asked about the root cause for patterns of hypoactivity and the extent to which they are intrinsic to autism, per se. The subclinical condition describing difficulties in emotional awareness (alexithymia) is present in 10 % of the general population, compared to 50 % of high-functioning autistic individuals. As such, neural patterns attributable to comorbid conditions should be suspected. Strong relationships are observed between alexithymia and metrics of empathy, indicating a link between understanding one's own emotions and understanding the emotions of others. When asked to introspect on one's own emotions, diminished activity of the anterior insula is most associated with the degree to which alexithymia is reported. Empathy scores also correlated significantly with bilateral mid-anterior insulae in both the autism and the control groups. Taken as a whole, it may be possible that difficulties of autistic individuals in responding to emotional cues from bodily gestures might be at least partially explained by confounding conditions (Silani et al. 2008).

In exploring the mechanisms for emotional processing in autism, another entry point is to explore responsiveness to facial communication of feelings. Tasks in which volunteers encounter facial cues of emotional expressions consistently report hypoactivity of functionally pertinent regions in autistic individuals in comparison to control subjects exposed to the same stimuli and tasks (Bolte et al. 2006; Ashwin et al. 2007; Loveland et al. 2008; Philip et al. 2010). When subjects are asked to assess congruence in the emotions represented by different faces, typically developing individuals demonstrate significantly greater recruitment of multiple brain regions, including the orbitofrontal, superior temporal, parahippocampal, occipital, and cingulated cortices (Loveland et al. 2008). Perception of fearful faces has been shown to specifically activate the left amygdala and the left orbitofrontal cortex more pronouncedly in control subjects than in autistic volunteers (Ashwin et al. 2007).

Adding insight toward the role of the amygdala in difficulties with emotional facial processing, there is significantly greater amygdala habituation bilaterally in control subjects than in autistic individuals. In contrast, there are no observed differences in fusiform habituation between control and autism groups. For autism spectrum disorders, lower levels of habituation of amygdala to face stimuli are associated with more severe social impairment, further pointing toward amygdaloid dysfunction in aberrations related to facial emotional processing (Kleinhans et al. 2009).

Taken as a whole, one wonders whether deficiencies in emotional processing in autism are most appropriately considered to be task specific or stemming from a unifying, high-level deficiency in cognitive processing particular to emotion. There seems to be evidence that could be interpreted to support either claim. Whereas certain behavioral and neural deficiencies are clearly task specific (Piggot et al. 2004), other findings demonstrate emotionally related abnormalities than span sensory modalities, indicating the possibility of higher-level aberrations in processing emotion (Philip et al. 2010).

The question arises whether some of the differences in neural patterns associated with emotional processing in autism are reflective of alternate neural strategies for interpreting and responding to emotional input. In other words, to what degree are differences in neural function indicative of alternate and compensating cognitive styles? In the perception of fearful faces, for example, autistic participants demonstrate reduced amygdaloid activity. But they also show greater intensity in the response of anterior cingulate and superior temporal cortical regions of the brain (Ashwin et al. 2007). In a study examining the comprehension of sentences with multiple meanings, participants with autism showed an overall increase in neural activation compared to neurotypical individuals. Results indicated that individuals with autism resort to altered neural routes compared to typically developing individuals in certain cognitive tasks (Kana and Wadsworth 2012). Further corroborating the use of alternative and compensatory neural mechanisms in persons with autism, a computer-based training module was implemented to teach facial affect identification to children with autism. The training significantly improved task performance; but neural correlates for post-training improvements did not correlate to anticipated neural regions, such as the fusiform gyrus. Instead, neural activity

increases were observed in the superior parietal lobule and the medial occipital gyrus, suggesting alternate neural routes employed for accomplishing normative behavioral expectations (Bolte et al. 2006).

It is worth noting that emotional, facial, and social processing represents relatively high-level visual perception (faces) and high-level cognitive processing (social reasoning). It has been demonstrated, for example, that systems for recognition of objects and places (comparatively simple computational tasks) mature earlier than systems for face processing (Humphreys et al. 2008). In this light, there is commonality in the relative level of computational sophistication required for these respective tasks. As such, it is unresolved whether abnormalities in face, social, and emotional processing are distinctly derived from deficiencies in specific functions or whether the cognitive difficulties specific to autism are emergent from the increased complexity of the tasks corresponding to dysfunctions typifying autism spectrum disorders.

10.11 Attention and Working Memory

The attention control network is composed of a distributed network of brain regions that includes bilateral intraparietal sulcus, bilateral frontal eye fields, bilateral area V5/MT, bilateral dorsolateral prefrontal cortex, anterior insula, and anterior cingulate regions (Corbetta and Shulman 2002). This network may be subdivided into a dorsal frontoparietal attention network (intraparietal sulcus and frontal eye fields along the superior convexity), a more lateral ventral attention network (dorsolateral prefrontal cortex, lateral intraparietal sulcus, and superior temporal sulcus), and a salience or novelty detection network comprising bilateral anterior insula and dorsal anterior cingulate cortex (Fox et al. 2006; Seeley et al. 2007) (Fig. 10.3).

In a group of studies examining activation patterns for individuals with autism engaged in task that stimulate these attentional networks, the findings are more variable and less striking than in other domains reviewed above. This may be in part due to relatively small sample sizes of the studies, with most of the studies including less than ten subjects per group. In an oculomotor task where subjects had to remember the location of a stimulus and shift their gaze to the remembered location following a delay, Luna and colleagues found decreased activation for autism participants in small loci in the dorsolateral prefrontal cortex and posterior cingulate cortex (Luna et al. 2002). Belmonte et al. used a covert visual attention-shifting task and found increased activation in autism along the left intraparietal sulcus but decreased activation in the ventral occipital cortex (Belmonte and Yurgelun-Todd 2003). Increased activation in frontoparietal attentional regions in autism was also seen in a visuomotor sequence task performed by Muller and colleagues (2003).

Koshino and colleagues used an n-back task where subjects had to remember whether a stimulus was the same as a prior stimulus in a sequential list (Koshino et al. 2005). They found greater right lateralization of attentional regions in autism with greater activation of occipital regions in autism. Haist et al. used a task requiring maintenance of a visuospatial cue and reported decreased activation in autism in

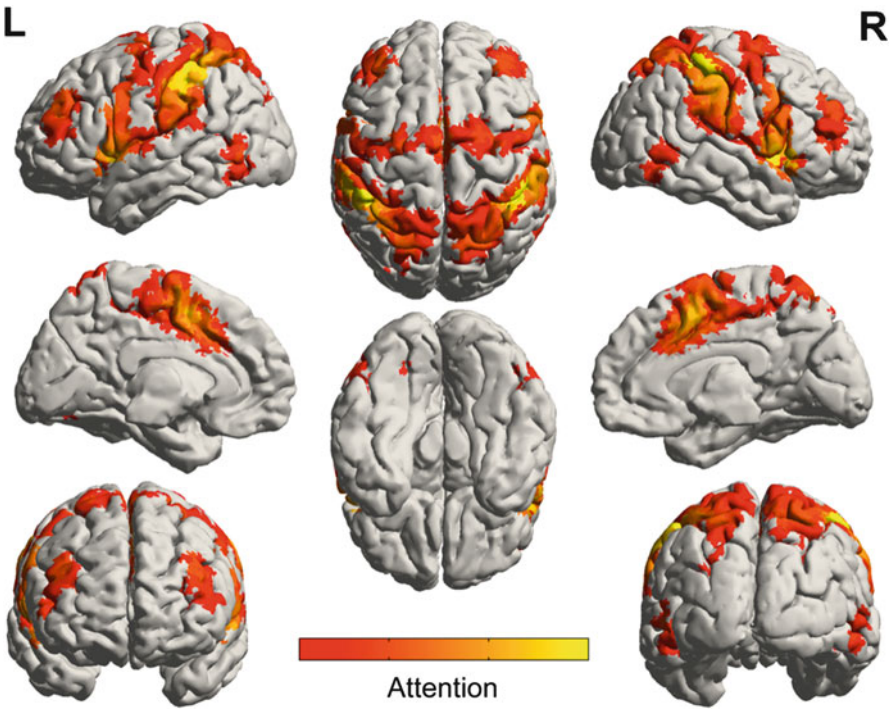

Fig. 10.3 Core attentional regions in the brain. The images represent areas that are synchronized in resting state fMRI images to four seeds in the bilateral intraparietal sulcus and bilateral anterior insulae across 1,353 typically developing subjects. Included regions are bilateral intraparietal sulcus, area V5/MT, dorsolateral prefrontal cortex, dorsal anterior cingulate, lateral premotor, and frontal eye field cortical areas

scattered regions including the cerebellum, frontal, parietal, and occipital lobes, with the biggest difference in the inferior parietal lobule (Haist et al. 2005). In a study evaluating responses of both autism children and unaffected siblings to attended visual stimuli, both siblings and children with autism showed abnormal activation of the prefrontal cortex (Belmonte et al. 2010).

In studies with somewhat larger sample sizes, Bird and colleagues instructed participants to attend to face or house visual stimuli and found decreased attentional modulation of fusiform face area but not parahippocampal place area, suggesting impaired attention to faces but not houses (Bird et al. 2006). Redcay and colleagues had participants play a game where they attended to a location in a cartoon house where a mouse was hiding. For trials where they attended to the same area as a joint observer with which they were interacting, there was reduced activation of the posterior superior temporal sulcus relative to control subjects (Redcay et al. 2012). Christakou et al. used a sustained vigilance task that found decreased activation in dorsolateral prefrontal, superior parietal, and striatothalamic regions in cohorts of autism and attention deficit-hyperactivity disorder (ADHD) boys (Christakou et al. 2013). They also found decreased deactivation of the default mode network in

autism. Ohta and colleagues did not find differential activation of visual attention in autism in a visual target detection task, but found an abnormal modulation of visual attention in the presence of distractors (Ohta et al. 2012).

10.12 Executive Function and Response Inhibition

Related to the attentional or working memory network discussed above is a network of brain regions responsible for response inhibition and control of executive function. These functions also involve dorsolateral prefrontal cortex as well as more orbitofrontal cortex, anterior cingulate, and right intraparietal sulcus.

Schmitz and colleagues analyzed brain activation patterns during three separate executive tasks: a go/no-go task where subjects either made a response or refrained from response depending on a cue, a Stroop task where conflicting information must be processed, and a set shifting task. Autism subjects showed increased activation of left inferior frontal and orbitofrontal gyrus, left insula, and areas within the parietal lobes (Schmitz et al. 2006).

A go/no-go task was also employed by Kana et al. (2007), who found decreased activation in autism in the anterior cingulate cortex, and Goldberg et al. (2011) who found reduced deactivation of medial prefrontal and left superior temporal sulcus during the commission of errors in response.

A Tower of London task was used in by Just et al., who found no difference in activation between autism and control samples but did report decreased frontoparietal functional connectivity in autism (Just et al. 2007). In a task measuring cognitive control, where subjects made responses to a cue in the presence of distracters, Solomon et al. found decreased recruitment in autism of frontal, parietal, and occipital regions for more difficult trials compared to control subjects (Solomon et al. 2009).

Agam and colleagues reported decreased activation of the frontal eye fields and anterior cingulate cortex in autism in a response inhibition task involving oculomotor control also reviewed in the section on gaze control (Agam et al. 2010). Gilbert et al. used two tasks including a task where subjects reported features of alphabet letters that were either visualized or imagined (Gilbert et al. 2008) and found reduced deactivation of the medial prefrontal node of the default mode network.

This group of studies does not appear to show any clear pattern of activational differences in autism and resembles results of functional connectivity in autism in the attention control network where abnormalities are much less salient than in other brain networks. Moreover, these are tasks for which autism subjects do not show consistent behavioral deficits as with motor, language, and social functioning.

10.13 Reward, Salience, and Novelty

Another related subnetwork of the attention control network is the salience or novelty detection network (Seeley et al. 2007). Brain regions in the bilateral anterior insula and dorsal anterior cingulate typically show activation when an individual

encounters a novel or salient stimulus regardless of the stimulus modality. Such stimuli can also result in activation of dopaminergic pathways involving the dorsal striatum and nucleus accumbens and for stimuli with affective content, the amygdala. Such stimuli are particularly relevant to autism given the well-known "resistance to change" or "insistence on sameness" phenotype wherein individuals with autism may become irritated or anxious when encountering unexpected events or changes to routine (Baron-Cohen 2006).

The first characterization of functional brain responses to novel stimuli was by Gomot et al. (2006) who used an auditory oddball task where subjects passively listened to novel sounds from among the more frequently presented standard tones while watching video clips. Autism subjects showed reduced activation in response to novel stimuli in the anterior cingulate cortex as well as bilateral temporoparietal regions and right inferior and middle frontal regions. A subsequent study in which subjects were actively required to listen for novel stimuli resulted in increased activation in autism in areas of prefrontal, premotor, and left inferior parietal cortex (Gomot et al. 2008).

A target detection task (set shifting) where subjects classified stimuli as "target" or less frequently "nontarget" based on the shape of the object (Shafritz et al. 2008), autism subjects showed reduced activation in frontal, striatal, and parietal regions, with largest clusters of differential response in the right dorsolateral prefrontal cortex and left putamen.

In a study where participants were given monetary rewards, autism participants showed increased brain activation of the left anterior cingulate gyrus when rewarded relative to control subjects, with linear relationship to social impairment (Schmitz et al. 2008). But in a subsequent study also involving monetary rewards, autism participants showed hypoactivation of the nucleus accumbens for monetary stimuli and hypoactivation of the amygdala and anterior cingulate cortex for both monetary and social stimuli (Kohls et al. 2012). Similarly, during a rewarded implicit learning task, autism subjects showed decreased activation for both social and monetary rewards in the striatum and anterior cingulate cortex (Scott-Van Zeeland et al. 2010a), and in a study measuring responses to faces, monetary rewards were associated with relative hypoactivation of right nucleus accumbens (Dichter et al. 2012a, b).

In considering the results obtained for executive, attentional, and reward tasks, autism subjects do not appear to show global decrease in activation in attentional and executive networks, but more variable, task-specific, or study-specific findings. For simple novelty tasks, there was variable activation of the salience network in autism that was modulated by subject arousal or attention. Yet, when monetary or social rewards were provided, autism subjects seem to consistently show hypoactivation of corticostriatal reward circuitry across several studies.

10.14 Imitation and the Mirror Neuron System

Considerable progress has been made in establishing the brain regions involved in discriminating self from other. In particular, specialized circuits appear to be involved in mimicking or mirroring the actions of another person that some have hypothesized

may be a precursor to empathy perception. When subjects perceive and imitate a hand motion of another person, there are brain regions that are similarly active whether the movement is performed by oneself or by another (Iacoboni et al. 1999). These regions are seen in the premotor cortex along the mouth of the operculum and in the anterior inferior parietal lobule (Cattaneo and Rizzolatti 2009; Molenberghs et al. 2012) and have inputs from the amygdala and superior temporal sulcus (Iacoboni and Dapretto 2006). During imitation of facial expressions, activation in motor and premotor cortex, superior temporal cortex, and superior mid- and anterior insula is observed that is greater for imitation than merely observation of expressions (Carr et al. 2003), with the insula serving as a relay between mirror neuron systems in the frontal and parietal lobes and the limbic system. Given that individuals with autism show particular deficits in empathy and imitation (Williams et al. 2001), there has been suggestion that the mirror neuron system may be a locus of pathology for autism (Iacoboni and Dapretto 2006), a subject of an excellent review by Kana et al. (2011b).

During imitation of emotional expressions, Dapretto and colleagues found a modest decrease in activation in the ventral premotor region for autistic participants, providing early evidence that this region may be impaired in autism (Dapretto et al. 2006). During imitation of hand movements, Williams et al. found decreased activation in the inferior parietal cluster of the mirror neuron system in autism (Williams et al. 2006). In contrast, a small sample of subjects were studied by Martineau and colleagues during imitation of hand movements and found greater activation in autism in the inferior frontal cluster (Martineau et al. 2010). A novel approach by Dinstein et al. not only evaluated activation during imitated hand movements in autistic and control subjects but used the paradigm of fMRI adaptation to evaluate the differential responses for specific movements. They found no difference in selective activation of the mirror neuron system in autism (Dinstein et al. 2010). Using a task where subjects perceived rational and irrational hand movements, Marsh et al. found that the frontal and parietal mirror neuron regions activated normally in autism but that there was decreased activation in the anterior cingulate, supplementary motor area, and fusiform gyrus in autism (Marsh and Hamilton 2011).

Behaviorally, it has been noted that autism subjects often exhibit hyperimitation, including echolalia, and a study evaluating imitation and mentalizing tasks indicated that shared neural correlates of attribution of mental states and control of imitation may lie in the default mode network, particularly the medial prefrontal node, and this area may be abnormal in autism (Spengler et al. 2010). Taken together, these studies do not support a clear deficit in the mirror neuron system in autism but are rather more consistent with generalized deficits in processing in complex networks that are heterogeneous in autism and not specific to imitation.

10.15 Theory of Mind

Another set of experiments has investigated discrimination of self and other using more cognitive and mentalizing tasks. It is believed that typically developing individuals possess a “theory of mind” allowing the perception of mental states of self and

other. This function is subserved by a set of brain regions predominantly in the default mode network that focus attention on inner state or internal stimuli, dialogue, and narrative. There is considerable evidence that individuals with autism show impairment in this network, resulting in clinical deficits of "mindblindness" or impaired theory of mind (Baron-Cohen et al. 1985; Lombardo and Baron-Cohen 2011).

The default mode network has been one of the most consistently abnormal networks in neuroimaging studies of autism. Across a number of tasks during which the default mode network is typically deactivated, autism subjects fail to show such deactivation (Kennedy et al. 2006; Mason et al. 2008). The default mode network is often more active during control or rest blocks than during experimental manipulations, but there are a number of cognitive paradigms that directly activate this network.

Chiu and colleagues employed two fMRI tasks, a visual imagery task, where subjects watched clips of athletes and imagined participation to define "self" and "other" activation regions in the cingulate cortex, and a multi-round trust game involving monetary rewards. When playing the trust game with a human partner, autism subjects showed impaired activation of the cingulate gyrus in regions associated with "self" perception (Chiu et al. 2008). A mentalizing task by Kennedy and Courchesne required subjects to evaluate true or false statements about self or other that were based on either psychological (internal) or observable external characteristics. They observed a modest reduction in medial prefrontal activity across all judgment conditions (Kennedy and Courchesne 2008). During a task where geometric shapes "interacted" with each other either in a random or goal-directed fashion, autism subjects showed reduced activation in the medial prefrontal node of the default mode network as well as reduced functional connectivity between anterior and posterior nodes of the network (Kana et al. 2009). Lombardo and colleagues employed a task where subjects mentalized about opinion questions (e.g., "How likely are you to think that keeping a diary is important?") about either self or public figures. Neurotypical subjects activated the medial prefrontal region more for questions involving self than other, whereas autism subjects did not exhibit this distinction (Lombardo et al. 2010). In another study when subjects made either physical or mentalistic judgments about self or other, the right temporoparietal junction was specifically hypoactive during self-judgments in autism (Lombardo et al. 2011), with deficits showing linear correlation to social impairments on ADI-R testing. Considering several fMRI experiments, Bara concludes that autism and schizophrenia may be at two poles of a continuum where autism is hypo-mentalistic and schizophrenia is hyper-mentalistic (Bara et al. 2011).

These studies, when combined with the pervasive deficits and functional connectivity in the default mode network in autism reviewed in a separate chapter, are consistent with a network-specific impairment in the default mode network that may arise from long-range communications between internal and external attentive networks. As a consequence, the default mode network is not deactivated when subjects attend to the outside world, resulting in an overactive internal narrative. At the same time, there is both hypoconnectivity and impaired activation of particularly the medial prefrontal node of the network that may represent impaired ability to form robust percepts about the self, one's mental state, and self-other discriminations.

10.16 Learning and Abstract Reasoning

Individuals with autism exhibit impairments in abstract reasoning, particularly where information must be integrated from multiple sources, although autism subjects may excel in recognizing subtle nuances in details. Accordingly, investigators have examined brain activation during learning and analytical reasoning tasks in autism. In a task where participants were required to recognize sequences of six or eight digits, autism subjects showed increased activation in right premotor cortex (Muller et al. 2003, 2004), although sample sizes were small (5–8 subjects per group).

Studies have also examined learning using pictorial stimuli. In a study comparing pictorial to semantic reasoning, while similar brain regions were activated for autism and control samples, autism participants showed relatively greater activation for occipital and temporal clusters, while typically developing subjects showed greater activation for frontal language regions in performing the learning task (Sahyoun et al. 2010). In a task where subjects were required to learn prototypes from random dot patterns, autism subjects showed impaired generalization of learned prototypes to new conditions (Froehlich et al. 2012).

Behavioral studies have found that high-functioning autism individuals may perform certain reasoning tasks at levels exceeding what would be expected for IQ (Dawson et al. 2007). One such example is Raven's Standard Progressive Matrices. In fMRI results obtained while performing this task, autism subjects showed greater occipital activation, while control subjects showed relatively greater lateral prefrontal cortex activation (Soulieres et al. 2009).

When learning was placed in the context of a social judgment task of discriminating video clips of lying actors from truth-telling actors, autism subjects showed a similar pattern of occipital and language region activation to control subjects, but did not show the same decreases in activation with learning that were seen in control subjects (Schipul et al. 2012).

In a moral reasoning task where 30 subjects per group answered questions involving social or ethical dilemmas, compared to more mundane problems without ethical implications, both groups activated classical regions of the default mode network but with higher activation of the posterior cingulate cortex in autism. At the same time, less activation of the amygdala was seen in autism for social and ethical dilemmas (Schneider et al. 2012). This is particularly intriguing given the well-established failure to deactivate the default mode network in numerous other studies above as well as consistently decreased functional connectivity within the default mode network. In context of Schneider et al., the failure to deactivate the default mode network during attention to external stimuli may also exhibit greater recruitment of the default mode network for more compelling internal stimuli. In other words, the default mode network may be more tonically active in autism yet with decreased interregional synchrony that makes the network less efficient.

10.17 Brain Lateralization

Lateralization of some brain functions occurs in typical development (Toga and Thompson 2003). Language is an example of functional specialization lateralized to the left hemisphere in ~95 % of right-handed and ~75 % of left-handed typically developing individuals (Knecht et al. 2000a, b). Nevertheless, abnormal functional lateralization is characteristic of neurospsychiatric disorders, including autism (Herbert et al. 2002, 2005; Fletcher et al. 2010; Lange et al. 2010a). The earliest reports that suggest abnormal brain lateralization in autism are behavioral, structural imaging, and electrophysiological studies (Dawson et al. 1986; Hauck and Dewey 2001; Herbert et al. 2002, 2005; Escalante-Mead et al. 2003).

More recently, functional imaging studies have found abnormal lateralization in brain regions involved in language processing. Anderson et al. found decreased left lateralization in Wernicke's area compared to its homologue in adolescents and adults with autism compared to typically developing participants, during an auditory phrase-recognition task (Anderson et al. 2010). Two other fMRI studies have found that typically developing adolescents and adults have significant left lateralization in language regions of the frontal lobe, whereas adolescents and adults with autism either lack left lateralization or have reversed lateralization during language tasks (Kleinhans et al. 2008b; Knaus et al. 2008).

There are also reports of abnormal functional lateralization of language regions in children with autism. Redcay et al. report a trend of left-lateralized function in typically developing toddlers listening to speech stimuli while sleeping, whereas toddlers with autism lack any functional lateralization in Broca's or Wernicke's area (Redcay and Courchesne 2008). In another study of a larger sample size, the same lab confirmed the trends of atypical lateralization in the superior temporal cortex of toddlers with autism (Eyler et al. 2012). They also demonstrated that the effects worsen with age to a peak at 3–4 years (Eyler et al. 2012).

10.18 Endophenotypes, Biomarkers, and Classification

Early attempts at using fMRI for diagnostic or prognostic biomarkers have now been reported in the literature. Although the vast majority of reviewed studies evaluate populations, showing statistical differences in mean activation or patterns of activation, the application of fMRI for single-subject treatment decisions will require sensitive and specific information in a single subject's imaging data, a much more difficult test than has previously been attempted for fMRI.

In a study using multivoxel pattern classification, investigators analyzed the voxel-by-voxel pattern of activity in the medial prefrontal cortex during a mentalizing task where subjects reflected on another person's mental state (Gilbert et al. 2009). Even though no group differences in task activation were seen, the individual voxel-based pattern of activation differed between groups. While this study did not attempt identification of individual subjects, the method is intriguing that there

might be information locked within an fMRI scan beyond cluster-level activation that may be a useful biomarker. In another study using multivoxel pattern classification during a facial recognition task, the multivoxel classifier was significantly correlated with social impairment from ADOS measurements (Coutanche et al. 2011), again despite no differences in mean activation at the cluster level.

If MRI is to be used for diagnosis or prognosis in a clinical population, methods to assess the sensitivity and specificity of an assay are needed. Structural MRI has been used for classification in several studies. Uddin and colleagues used a searchlight algorithm to evaluate voxel-based morphometric data from a structural MRI scan to obtain an accuracy of approximately 90 % for distinguishing children and adolescents with autism from neurotypical controls (Uddin et al. 2011). Using a support vector machine-learning algorithm, Ecker and colleagues obtained similar accuracies (Ecker et al. 2010a, b). In a study using diffusion tensor imaging of the temporal stem, accuracies exceeding 90 % were also obtained (Lange et al. 2010b).

Recently, several laboratories have used functional MRI to attempt classification or endophenotyping of autism subjects, including imaging of unaffected siblings to evaluate whether functional imaging derived biomarkers may represent true endophenotypes. Belmonte and colleagues performed a visual attention task in controls, individuals with autism, and unaffected brothers of the autism participants to find that patterns of activation and functional connectivity in the frontal lobes and cerebellum could distinguish all three subgroups (Belmonte et al. 2010). Fathers of children with autism showed similar hypoactivation of the fusiform gyrus to autism subjects (Greimel et al. 2010). Spencer and colleagues evaluated autism participants and their parents during a phonological processing task found abnormalities in activation in the postcentral and supramarginal gyrus that matched the abnormalities seen in the autism children (Spencer et al. 2011). In a study using a biological motion task, unaffected siblings showed activation abnormalities in the posterior temporal and lateral prefrontal cortex that allowed distinguishing autism, siblings and controls (Kaiser et al. 2010).

Using a whole-brain functional connectivity analysis, Anderson et al. found that unaffected siblings behaved more like controls than autism participants, without identifying any intermediate state where siblings of autism subjects showed intermediate or disease phenotype (Anderson et al. 2011). In this study, single-subject diagnosis was attempted with accuracy around 80 % for all subjects and 90 % for subjects under 20 years of age (Anderson et al. 2011).

These studies are yet preliminary without replicated phenotypes that have sensitivity, specificity, and across-site reproducibility that are suitable for application to a target clinical population but show feasibility of obtaining such a clinically useful test. At present, neither structural nor functional imaging is clinically helpful, beyond exclusion of other disorders such as tuberous sclerosis, neurofibromatosis, or developmental brain malformation (Lauvin et al. 2012). Application of MRI-based biomarker and classification methods to younger cohorts will be important to provide diagnostic or accessory prognostic information at an age where this data can constrain choices for interventions. Adoption of imaging biomarkers for clinical screening will also require multisite replication studies to confirm that such complex tools can be widely applied, at least in tertiary imaging centers, with interpretable results.

10.19 Conclusions

Task-related fMRI studies provide a useful tool for comparing deficits in autism associated with regional brain activation and different neural subsystems. The literature is heterogeneous not only in the types of brain functions studied, resulting group differences, and technical features of the studies including sample size and methods. Results illustrate that brain abnormalities in autism are not confined to any particular constellation of brain regions. Nevertheless, some clear trends emerge from the analysis across neural system and across task paradigms.

First, deficits are more often observed in association cortex than in primary sensory cortex. Most studies involving auditory, visual, and somatosensory cortex that find deficits show greatest impairment in modulation of sensory processing or second-order impairments in unimodal association cortex. Deficits in speed of processing in the earliest sensory perception region may be present, but it is likely that these deficits compound with successive stages of neural processing as perceptual information is propagated to higher-order cortical regions.

Second, brain activation deficits in autism are greatest for tasks and networks requiring integration of information from distributed networks. A prototypical example is the default mode network, which draws abstract information about the self from a variety of brain regions and forms perhaps the highest-order cognitive processing region in the brain. This network overlaps with the "social brain" which also draws information from widely separated regions such as superior temporal sulcus, mirror neuron system, language regions, and default mode network. Social brain tasks and regions also commonly show activational differences in autism.

Third, deficits are more likely to be observed in tasks where autism subjects exhibit behavioral impairments. Functions for which individuals with autism show relatively intact function such as visual search, matrix reasoning, and embedded figure tasks more often show mixed or weak differences in autism. In contrast tasks where autism subjects perform poorly, such as facial perception, oculomotor control, motor function, and biological motion perception, exhibit more consistent and pronounced differences in fMRI studies.

Fourth, most of the time that activational differences are seen in the autism brain, they represent hypoactivation of brain regions. Although there are notable exceptions, it is far more common for a study to report that a relevant brain region shows less activation.

Fifth, lateralized brain functions show increased recruitment of the contralateral hemisphere simultaneously with variable hypoactivation of the preferred hemisphere. This corresponds to established differences in autism showing weaker hand preference and more common bilaterality in neuropsychological testing.

This pattern of findings is consistent with a theory of autism as an underconnectivity or undersynchronization syndrome where abnormalities may be distributed throughout the brain rather than regionally specific and where function is most impaired in neural systems requiring integration of multiple discrete loci of information or synchronization of disparate networks. Higher-order cognitive functions

requiring multiple layers of abstraction are more impaired than tasks where a single brain region or domain is active.

Many open questions remain. Can brain activational differences account or help in subtyping autism or account for the heterogeneity of clinical features? Can fMRI be a useful adjunct to neuropsychological and clinical metrics that may help prognosticate or constrain treatment options? How do the numerous predisposing genetic markers that have been published correspond to brain activational differences? Are these genetic variants specific for deficits in neural systems, or is there a final common pathway for autism that is largely independent of the specific constellation of genetic abnormalities seen? Can abnormalities in brain activation predict with sufficient sensitivity and specificity clinically useful discriminations at an early enough age to impact clinical diagnosis? Given that the summary picture in autism is one of nuanced changes in interregional activation patterns rather than architectural changes in the brain, can other techniques with finer temporal resolution such as EEG and MEG discriminate at finer temporal scales the specific timing differences that may underlie impaired synchronization?

Despite limitations and extensive room for debate on the interpretation of the autism functional imaging literature, there is convergent information that imaging can be a useful marker for the disease and that constraints can be placed on brain abnormalities that inform theories of autism pathophysiology in ways that would not be possible without such an fMRI literature. The field of functional imaging of autism continues to accelerate and differentiate, and there is great cause for optimism that with increased sample sizes, more technical methodological sophistication, and the benefit of specific hypotheses from these early studies, the future work will be increasingly effective at characterizing specific brain abnormalities that may allow the next generation of autism patients and families to take advantage of therapies designed to address the neurobiological underpinnings of the disease.

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Biography

Jeffrey S. Anderson received his M.D. and Ph.D. degrees from Northwestern University in neuroscience. He completed residency training in diagnostic radiology and a neuroradiology fellowship before joining the faculty at the University of Utah School of Medicine. He directs the fMRI neurosurgical mapping service, and is principal investigator for the Utah Functional Neuroimaging Laboratory. Dr. Anderson's lab studies brain networks using functional imaging techniques, with particular interest in autism.

Michael A. Ferguson completed his Bachelor of Science degree in biochemistry at Brigham Young University before studying medicine at the State University of New York. He is currently working on his Ph.D. in bioengineering, specializing in functional imaging. His research interests include the neural underpinnings of social dynamics.

Jared A. Nielsen graduated from Brigham Young University with a bachelor's degree in neuroscience. He is currently pursuing a Ph.D. in neuroscience at the University of Utah. His dissertation work focuses on the lateralization of structural and functional networks and their behavioral correlates in autism.

Chapter 11
Functional Connectivity MRI in Autism

Jeffrey S. Anderson

11.1 Introduction

The theory underlying functional connectivity MRI was established in a landmark paper by Bharat Biswal and colleagues (1995). By obtaining serial images of the brain in the absence of any cognitive task, they observed synchrony of intrinsic blood–oxygen-level-dependent (BOLD) signal fluctuations in the primary motor cortex that recapitulated a network seen on motor task-performance studies. Since then, there has been an explosion of interest categorizing a new neuroanatomical paradigm where distributed networks of widely separated but functionally related brain regions have been characterized (Fox and Raichle 2007) (Fig. 11.1).

The use of functional connectivity methods to study autism has been among the most enthusiastic applications of fcMRI, with over 50 reports of abnormalities in autism in the last decade. Several excellent reviews have now characterized the abnormalities reported in these early studies (Kana et al. 2011; Muller et al. 2011; Schipul et al. 2011; Vissers et al. 2012). In this chapter, a review is presented of reports of abnormal functional connectivity with an emphasis on describing areas of convergent abnormalities across studies and assessing whether the literature can support a unified theory of brain connectivity underlying autism.

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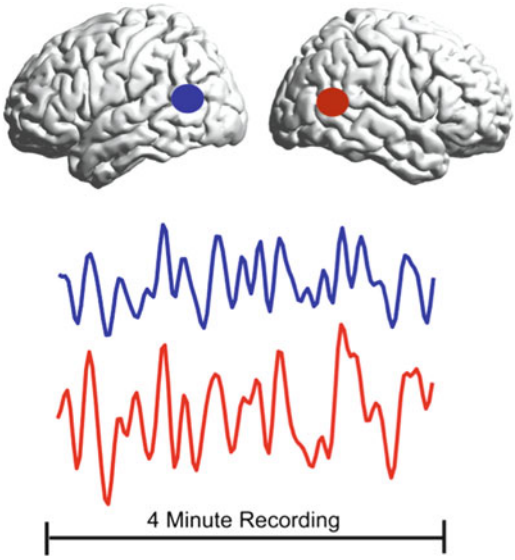

Fig. 11.1 Synchronized BOLD recordings. The traces below were obtained from *left* (*blue*) and *right* (*red*) temporo-occipital cortex as shown in the images, representing a 4-min recording of BOLD signal, while the single subject rested with their eyes open in the MRI scanner. Even though the traces are obtained from opposite hemispheres when no task was present, there is strong synchrony between the two regions

11.2 Decreased Functional Connectivity in Autism

The initial report of decreased functional MRI connectivity in autism was published in 2004 by Marcel Just and colleagues (2004), with related reports of decreased connectivity in autism in different neural subsystems by two independent groups shortly thereafter (Villalobos et al. 2005, Welchew et al. 2005).

Just and colleagues presented a series of 17 high-functioning autism subjects, compared with 17 healthy typically developing subjects, in which they examined BOLD fMRI data acquired during a sentence comprehension task. Sentence comprehension blocks were interspersed with eight 24-s "resting" intervals during which subjects visually fixated on an asterisk on the screen, which were excluded from the analysis. Regions of interest (ROI) were selected for which a significant difference in activation was observed between task and control epochs. For each of these ROIs, a time course for the region was extracted from the images, and correlation between the time series (synchrony) was measured for each subject. In ten separate pairs of ROIs showing activation by the task, the synchrony was greater for control subjects than for autism subjects (Just et al. 2004).

Although this observation was made from data obtained during performance of a language task and findings may result to differential task performance rather than any underlying structural connectivity difference, a pattern of widely decreased functional connectivity was established that has now been replicated in many but not all of the studies examining functional connectivity in autism, which will be discussed by neural subsystem below.

11.3 Connectivity in the Default Mode Network

One of the most compelling networks demonstrating decreased connectivity in autism is the default mode network. This network is particularly compelling in autism, because behaviors associated with these brain regions (internal stimuli, internal narrative, self-focus) correspond to symptoms of autism in which individuals may exhibit internal reflection at the expense of awareness of the outside world. Regions of the default mode network also comprise core regions ascribed to the brain's "theory-of-mind" network associated with self-identification and implicated in autism symptomatology (Baron-Cohen 1995). At least ten studies have examined connectivity or function within the default mode network in autism (Fig. 11.2).

By pooling subjects from prior datasets using multiple different tasks, Cherkassky and colleagues amassed 57 autism and 57 matched control subjects and looked at the resting epochs for all of the tasks. They found that in 94 % of the pairs of 12

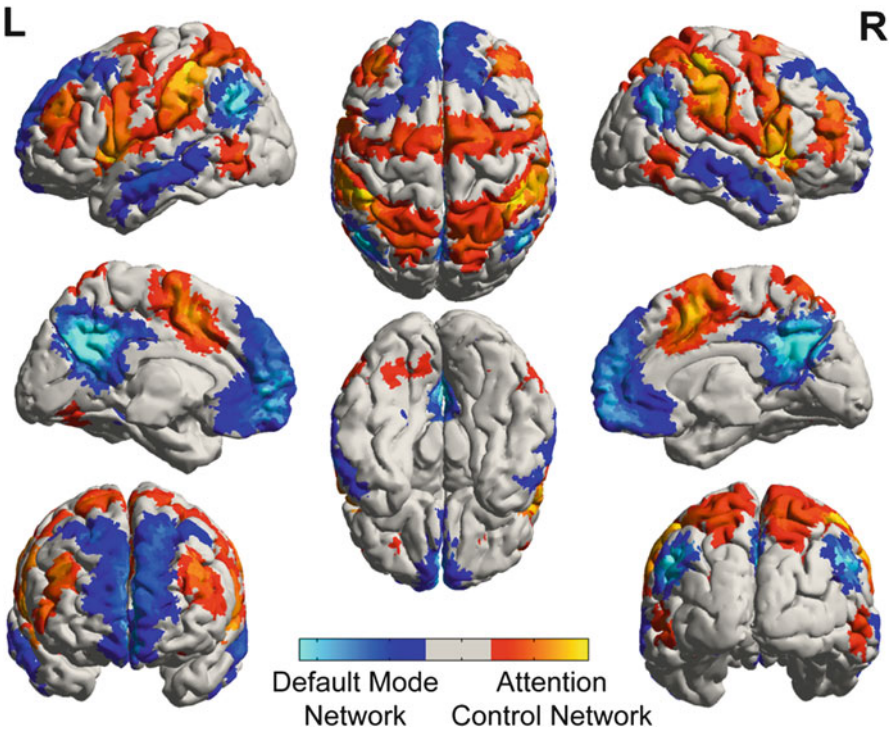

Fig. 11.2 Default mode and attention control networks. The images represent average functional connectivity to four seeds in each network (default mode: posterior cingulate, medial prefrontal, bilateral temporoparietal junction; attention control: bilateral intraparietal sulcus and frontal insula). The default mode network is shown in *cool colors* and the attention control network is shown in *warm colors*

regions comprising the default mode network, autism subjects had lower functional connectivity.

A contemporaneous study by Kennedy et al. took a different approach (Kennedy et al. 2006). They examined data from autism and control subjects during performance of a Stroop task where subjects were instructed to count words on the screen that were incongruent with the words shown (e.g., "two"). The investigators looked specifically at whether the default mode network regions deactivated during the interspersed resting (fixation) blocks compared to the task and found significantly less deactivation in autism subjects. A subsequent study (Kennedy and Courchesne 2008b) found that the attention control network did not show decreased connectivity in autism, while the default mode network did. A task-based study employing asking subjects to answer true/false questions about self vs. another person found reduced activity in the anterior node of the default mode network (Kennedy and Courchesne 2008a).

The relationship between the default mode network and theory of mind was probed directly by Mason and colleagues (2008) in a paradigm where subjects read passages requiring inferences about internal or emotional states vs. physical causality. They found increased recruitment of the right hemisphere for this task in autism, in particular the right inferior parietal lobule, whereas control subjects activated this region only for inferences about intentional states. The autism group also showed reduced functional connectivity within nodes of the default mode network.

In a study by Kana and colleagues (2009), participants viewed animated images and made judgments about mental states of the characters. They found decreased connectivity during the task between anterior and posterior nodes of the default mode network and hypofunction of theory-of-mind regions that correlated to decreased performance on psychometric testing of theory-of-mind function.

This finding of decreased anterior/posterior default mode connectivity was independently reproduced in a resting study by Monk and colleagues (2009) that correlated with poor social function. A similar study in adolescents confirmed decreased connectivity associated with 9 of 11 default mode network regions in autism subjects, with weaker connectivity associated with both poorer social function and higher restricted interests and repetitive behaviors on questionnaires (Weng et al. 2010).

Using a separate analysis technique of independent component analysis, Assaf and colleagues also find significantly weaker functional connectivity between nodes of the default mode network that was correlated with poorer social function on objective and subjective testing (Assaf et al. 2010).

Finally, in a study wherein participants made mental or physical judgments about self vs. others, autism subjects showed relative hypoactivation of the medial prefrontal cortex DMN node with weaker functional connectivity to several other brain regions and medial prefrontal cortex, also correlated with disease severity (Lombardo et al. 2010).

These studies show virtually uniform decreases in internal connectivity within the default mode network associated with hypofunction of particularly the anterior, medial prefrontal node and correlated with disease severity. Nevertheless, as is typical for fMRI studies, results are often in context of failure to deactivate during a task or decreased contrast between two mental states. Many of these results could also

be obtained if autism subjects showed tonically greater activity in default mode network regions, although this would be harder to explain in studies without an underlying task. Alternatively, it is possible to explain all of these results not by weak connectivity within the default mode network but rather by overconnectivity between attention control network regions and default mode network regions. Since the default mode network and attention control network exhibit to some extent an inverse relationship in temporal activity profiles (Fox et al. 2005), increased correlation between the two networks may result in weaker segregation of the networks and decreased synchrony between DMN nodes. Further studies may clarify the relationship of functional connectivity between DMN nodes and areas outside the network, such as the attention control network.

11.4 Connectivity in Facial Processing

Functional connectivity in autism for brain regions associated with face processing has been evaluated in many task-based and several functional connectivity studies. It is widely believed that individuals with autism have difficulty or disinterest in visualizing faces, resulting in considerable interest in the neural mechanisms underlying these deficits.

A study by Welchew and colleagues (2005) scanned 26 subjects while viewing fearful facial expressions and found decreased synchrony of the amygdala and parahippocampal gyrus. Similarly, Kleinhans et al. (2008) scanned 40 subjects during a face identification task and found decreased synchrony of the fusiform face area and both the amygdala and posterior cingulate cortex, with reduced FFA–amygdala connectivity associated with poorer social function.

A study by Bird et al. (2006) used the technique of dynamic causal modeling to compare differences in activation in response to face or house visual stimuli when the stimuli were attended to or not attended to. The results indicated decreased top–down attentional modulation of primary visual cortex to extrastriate visual cortex information processing, selective for faces. Given the potential for an attentional bias, Monk and colleagues (2010) used reaction times to control for attentional differences on trials and found that ASD subjects showed greater activation of the right amygdala to emotional faces than TD subjects. Functional connectivity between right amygdala and the anterior temporal lobe was weaker, while amygdala to medial prefrontal cortex was stronger.

11.5 Connectivity in Visual Search and Perception

Several studies have now examined functional connectivity in association with complex visuospatial perception tasks. The domain of visual perception, unlike complex social or empathic brain networks, is often seen as relatively spared or even enhanced in autism. Villalobos and colleagues measured decreased functional

connectivity in autism between primary visual cortex and inferior frontal cortex (Brodmann area 44) during a visuomotor task where participants pressed a button following a visual cue (Villalobos et al. 2005). A study obtained during a coherent motion paradigm by Brieber et al. showed no difference in functional connectivity between V1 and V5 or between left and right V5 in autism (Brieber et al. 2010).

One set of experiments used imaging during an embedded figure task where participants were instructed to find "hidden" shapes within a larger, more complex shape. Damarla et al. (2010) found that even though performance did not differ between the groups, there was reduced activation and functional connectivity among frontal and parietal attentional areas than for controls. In a separate study using a different embedded figure task that incorporated distracting information from a three-dimensional stimulus, Liu et al. found that activation patterns for autism subjects differed in that they did not see increased activation for the task in superior and medial frontal regions (Liu et al. 2011). They interpreted this finding as less difficult for the task in autism subjects, possibly because the 3D distracters were less confusing for autism subjects. They observed corresponding decreased functional connectivity between medial frontal and posterior visuospatial ROIs.

Intriguingly, a subsequent study with larger sample size looked at data acquired during a visual search paradigm. After particularly rigorous motion correction that removed individual volumes showing motion for each subject, the data showed increased connectivity between the ventral and dorsal attention networks (subnetworks of the attention control network) and the visual network (Keehn et al. 2012). In a combined study using fMRI to define language and visuospatial ROIs and DTI to measure structural connectivity between the ROIs, autistic subjects also had preferentially higher connectivity between visuospatial regions, but lower connectivity for connections involving frontal language areas (Sahyoun et al. 2010).

Thus, while studies examining functional connectivity in the default mode network appear to show relatively uniform decreases in synchrony between network nodes, studies involving complex visual perception are mixed. During facial processing tasks, connectivity appears decreased both in attentional and visual association cortex. Yet during visual search or abstract feature recognition tasks, autism subjects showed increased functional connectivity between brain attentional regions. This may correspond to performance differences in tasks for which autism subjects excel vs. perform poorly. Alternately, there may be heterogeneity across brain networks in the extent to which connectivity is impaired in autism, consistent with the preserved connectivity seen in the attention control network despite impaired connectivity in the default mode network seen in the results of Kennedy and Courchesne (2008b).

11.6 Connectivity in Executive, Working Memory, and Attentional Regions

A number of studies have now directly examined connectivity during tasks associated with brain attentional, working memory, and executive regions. The first study to examine connectivity directly within the attention control network in autism was

by Koshino et al. (2005). Using an n-back task with letters, they found that the left inferior parietal lobule was less synchronized with the rest of the attention control network. A slight increase in right frontoparietal synchrony in autism was not significant. In a second experiment, Koshino et al. (2008) performed an n-back task with face stimuli. They found decreased functional connectivity in autism between frontal regions activated by the task and the bilateral fusiform areas. Frontoparietal connectivity in autism was lower, but this was not statistically significant.

Using a Tower of London task, Just et al. found that frontoparietal functional connectivity was lower in autism, with the regions tested lying more within the ventral attention network (Just et al. 2007). A cognitive control task performed by Solomon et al. (2009) consisted of a task where participants had to remember a cue (colored square) for 8 s and then make a response to an arrow stimulus based on the color of the cue. Since a minority of responses required incongruent responses, the task was also a response inhibition task. Functional connectivity analysis showed reduced frontoparietal connectivity within the attention control network (Solomon et al. 2009). In a study that limited analysis to the frontal eye field and anterior cingulate cortex using an eye movement (saccade to target), task, performance, activation, and ACC–FEF functional connectivity were reduced in the autism sample (Agam et al. 2010).

Several studies have looked at regions associated with stimulus salience or novelty detection including the anterior insula and cingulate cortex (Seeley et al. 2007), a network also associated with response inhibition. In a go–no go task performed by Kana et al. (2007), participants were instructed to press or not press a button in response to a cue, with a minority of cues requiring no action. This task has design similarities with oddball tasks where the "salient" stimuli, those requiring no action, activate brain regions of the salience network. They found decreased responses in the middle cingulate cortex for autism subjects and decreased functional connectivity between cingulate cortex and right inferior parietal lobule. In a similar task in children, Lee et al. found a trend toward decreased functional connectivity between the right frontoinsular and right inferior parietal cortex (Lee et al. 2009).

Few studies have examined connectivity in autism within the attention control network using a resting paradigm. In a resting fMRI study of 25 typically developing adults, Di Martino and colleagues found a significant inverse relationship between cinguloinsular functional connectivity and Social Responsiveness Scale measurements, indicating that traits that resembled the autism phenotype within a neurotypical population are associated with decreased connectivity in the salience network (Di Martino et al. 2009). A resting-state fMRI study in adolescents showed decreased functional connectivity between the anterior and posterior insula (Ebisch et al. 2011).

In summary, connectivity studies within the attention control network show uniformly decreased functional connectivity in autism. Yet this observation could be almost entirely explained by differential task performance in autism subjects since almost all of the studies were performed in datasets obtained during an attentionally demanding task. Yet resting-state studies by Di Martino (Di Martino et al. 2009) and Ebisch (Ebisch et al. 2011) are not driven by external stimuli and also show decreased connectivity at least involving the anterior insula, a region that recurs in imaging studies of autism as abnormal (Uddin and Menon 2009). No studies to date have explicitly characterized connectivity between the attention control and default mode networks.

11.7 Connectivity in Language Regions

In addition to the initial report by Just and colleagues (2004) described above, functional connectivity in the language subsystem in autism has been examined in several subsequent studies. Kana and colleagues (2006) also performed scans during a sentence comprehension task but examined the imagery content in the sentences as a covariate. For high-imagery sentences, controls showed greater activation of language regions. Autism subjects exhibited a trend toward lower frontal–parietal functional connectivity.

A language memory task involving ten ASD and ten control participants evaluated functional connectivity between three seeds in the left occipital, left superior parietal, and left middle frontal regions and the rest of the brain. Increased connectivity between the seeds and scattered areas in the high medial posterior frontal, posterior temporal, and anterior occipital lobes was seen in autism (Noonan et al. 2009).

A study involving data collected during a verbal fluency task performed regression of task activation results and compared differences in connectivity attributable to differential task performance to differences in connectivity associated with spontaneous neuronal synchronization (Jones et al. 2010). Results showed that while functional connectivity involving seeds activated by the language task paradigm showed generally decreased connectivity in autism, this effect was greatest on the spontaneous interregional synchrony after task activity was regressed out of the data.

Given that autistic children sometimes make errors in deictic shifting (incorrect use of pronouns "I" and "you"), Mizuno and colleagues performed a study imaging participants during a linguistic perspective-taking task (Mizuno et al. 2011). Functional connectivity between the right anterior insula and the precuneus was decreased in autism, and within the autism group, this connectivity measurement was positively correlated with task performance (low reaction time). Lai and colleagues examined children during speech and song stimulation and found greater left frontal to left temporoparietal language connectivity during song than speech in autistic children, but no ASD/TD distinction could be made because many of the autistic children underwent propofol sedation for the exam (Lai et al. 2012).

A study by Dinstein and colleagues is the first to evaluate functional connectivity in nonsedated toddlers by imaging during natural sleep (Dinstein et al. 2011). The investigators regressed out task-evoked signals from auditory stimulation and evaluated interhemispheric correlation to find decreased synchrony in autism in the inferior frontal gyrus (Broca's area) and superior temporal gyrus (Wernicke's area) compared to controls. Inferior frontal gyrus interhemispheric correlation was negatively associated with communication and social deficits and positively associated with language function. Moreover, weaker interhemispheric correlations in inferior frontal and superior temporal gyrus could identify toddlers with autism with 72 % sensitivity and 84 % specificity (Dinstein et al. 2011).

11.8 Connectivity in Motor Regions

Individuals with autism typically have deficits in fine and gross motor skills, and a single study has evaluated functional connectivity within motor networks. Mostofsky and colleagues performed imaging during a finger-sequencing task and evaluated functional connectivity within bilateral motor cortex, bilateral cerebellum, bilateral thalamus, and supplementary motor area. Every pair of ROIs showed significantly greater functional connectivity in controls except for left vs. right cerebellum (Mostofsky et al. 2009). This corresponded with impaired behavioral performance on motor testing for the autism group.

11.9 Connectivity with Subcortical Nuclei

Studies of cortical/subcortical functional connectivity have examined both thalamocortical and corticostriatal synchrony. Mizuno and colleagues used data collected during a visuomotor coordination task to evaluate thalamocortical connectivity (Mizuno et al. 2006). They found more clusters in the cortex showing higher synchrony with the ipsilateral thalamus for their autism sample than for their control sample, although sample size was limited to eight subjects per group. The authors suggested that thalamocortical connectivity may differ from or be compensatory to the decreased corticocortical connectivity seen in autism in other studies.

Corticostriatal connectivity was evaluated during the same visuomotor task in eight subjects per group, and scattered areas of increased correlation to the left caudate were found in autism, while scattered areas of increased correlation to the right caudate were seen in controls (Turner et al. 2006). A subsequent study (Di Martino et al. 2011) examined 20 subjects per group with three seeds in each caudate and three seeds in each putamen during a resting-state acquisition. They found increased functional connectivity in autism between the striatum and areas of association cortex such as right superior temporal gyrus and bilateral insula as well as increased functional connectivity to the pons. One exception was decreased functional connectivity in autism between the striatum and posterior cingulate cortex.

These studies suggest a trend toward increased cortical–subcortical functional connectivity in autism that differs with most of the results seen for corticocortical connectivity. Several possible explanations might account for these differences. Subcortical connectivity may be to some extent compensatory for decreased corticocortical connections. If corticocortical networks are less robust in autism, then corticocortical connections may explain a lesser percentage of the variance of a cortical ROI's time series, and thalamocortical and corticostriatal "connections" may explain a relatively larger share of the cortical ROI's temporal fluctuations. Alternately, there are unique features about corticostriatal projections, namely, that direct pathway cortical–caudate projections are predominantly inhibitory (Rubchinsky et al. 2003), whereas corticocortical projections are typically excitatory. Thus, increased corticostriatal synchrony may actually represent decreased inhibition of the caudate by the cortex, still consistent with an underconnectivity hypothesis.

11.10 Local Connectivity

In its most commonly expressed form, the cortical underconnectivity hypothesis posits that autism is associated with short-range overconnectivity and long-range underconnectivity (Belmonte et al. 2004, Just et al. 2004). Yet the division between "short range" and "long range" can get substantially blurred as representing operationally a neuropsychological concept or graph–theoretical term between arbitrary nodes or physical definition about circuitry within a cortical column vs. local U-fibers vs. long-range projection fibers.

The most "short-range" type of connectivity that can be measured by techniques of functional connectivity MRI is synchrony between a voxel and its immediate neighbors, termed "regional homogeneity" (ReHo). Nevertheless this type of synchrony already extends over a scale of 3–5 mm and might seem already "long-range" from the perspective of an electrophysiologist or pathologist. Two studies have evaluated ReHo in autism. Paakki and colleagues measured regional homogeneity acquired during a resting state in adolescents and found a mixed distribution of intergroup differences. ReHo was decreased in autism for right superior temporal sulcus, right inferior and middle frontal gyri, bilateral cerebellar crus, right insula, and right postcentral gyrus, while ReHo was higher in autism for right thalamus, left inferior frontal, left subcallosal, and bilateral cerebellar hemisphere regions (Paakki et al. 2010). A second study by Shukla and colleagues acquired data during a visual search paradigm, with task parameters regressed out of voxelwise data. They found decreased ReHo in autism in superior parietal and anterior prefrontal regions, with increased ReHo in lateral and medial temporal regions, particularly on the right (Shukla et al. 2010). Both studies performed normalization of their ReHo measurements to control for noise, which limits the ability to detect global trends and predisposes toward a finding of a mixed spatial distribution of increased and decreased ReHo. Nevertheless, Shukla et al. (2010) performed an analysis without normalization and obtained similar results.

11.11 Connectivity in the Social Brain

There has been increasing attention in social neuroscience to a set of loci that are particularly active during fMRI studies of social function, empathy, and imitation of others, termed "the social brain." These regions include the anterior insula, superior temporal sulcus, amygdala, default mode, and language regions (Adolphs 2009). Several recent studies have begun to evaluate whether these regions or data acquired during social or emotive paradigms show particularly abnormal connectivity in autism.

Wicker and colleagues used a task paradigm where participants assessed the emotional valence of dynamic faces and evaluated the data using structural equation modeling for social brain loci (Wicker et al. 2008). They found weaker top–down effective connectivity between temporolimbic structures (amygdala, superior temporal sulcus) and prefrontal cortex. Results also showed increased effective connectivity between dorsolateral prefrontal cortex and fusiform face area.

A study examining data performed during letter detection and semantic decision tasks with task effects regressed out demonstrated increased connectivity between seeds in the right inferior frontal gyrus, superior temporal sulcus, and inferior parietal lobule with areas in the high medial prefrontal cortex and anterior cingulate cortex (Shih et al. 2010). Additional effective connectivity analysis using structural equation modeling showed decreased connectivity between inferior parietal lobule and inferior frontal gyrus in autism participants.

Decreased connectivity between socially relevant brain regions was also observed in a study by Schipul et al. from data performed during a social learning task where participants practiced detecting lying or truth-telling from facial expressions (Schipul et al. 2012). Not only did autism subjects show decreased connectivity between many of the 25 ROIs studied, but smaller increases in functional connectivity were seen during the learning period compared to the typically developing sample.

A recent resting-state analysis examined connectivity between social brain regions and found decreased functional connectivity in autism between nodes of the default mode network as well as between the amygdala and insula (von dem Hagen et al. 2012). Using both independent component analysis and seed-based approaches, von dem Hagen et al.'s study is one of few studies to also look at between-network connectivity in the case of the limbic network and the salience network, which was decreased in autism. A comprehensive analysis of social brain regions demonstrated widespread deacreased connectivity in autism (Gotts et al. 2012).

11.12 Distribution of Connectivity Abnormalities in the Brain

Most of the studies reviewed thus far have targeted specific neural subsystems to demonstrate differential connectivity in autism. The constellation of findings of generally decreased, though not uniformly decreased, connectivity suggests heterogeneity of connectivity differences that may not affect all brain regions equally. Two studies look at whole-brain connectivity differences to directly compare which region pairs show greatest synchrony differences in autism.

A study involving interhemispheric correlation examined resting-state data and compared each voxel with its interhemispheric homologue to assess a voxelwise difference in homotopic connectivity (Anderson et al. 2011c). Results showed heterogeneous connectivity that was weaker in autism for particular brain regions including the anterior insula, inferior parietal lobule, and posterior lateral frontal cortex, all predominantly association cortical regions.

In a study considering over 7,000 ROIs forming a lattice across the gray matter, functional connectivity was compared for every region pair, including over 26 million ROI pairs (Anderson et al. 2011d). This study found that functional connectivity among strongly synchronized, distant ROIs was generally weaker in autism but found that negatively correlated regions were less negatively correlated in autism. Although negatively correlated functional connectivity does not equate directly to underlying inhibitory connections (and may also represent a decrease in shared inputs from other sources), these results may be consistent with findings of

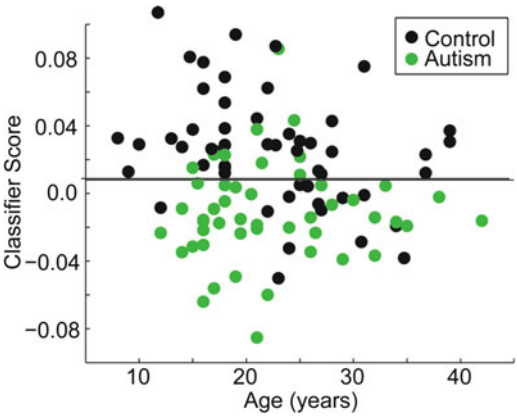

Fig. 11.3 Classification of typically developing and autism subjects using whole-brain functional connectivity MRI data. Accuracy was about 90 % for subjects under 20 years of age. Classifier score methods are described in Anderson et al. (2011a)

Di Martino et al. (2011) that showed increased connectivity in corticostriatal connections that presumably reflect a significant proportion of underlying inhibitory connections. Anderson et al. also found that regions most frequently involved in abnormal connectivity in autism were regions of the default mode network, fusiform gyrus, and anterior insula, all key regions implicated in the pathophysiology of social deficits of autism (Anderson et al. 2011d). The connectivity patterns were sufficient to classify autism with accuracy approaching 90 % for subjects under 20 years of age, where effects were largest (Fig. 11.3).

11.13 Behavioral Correlations and Modulating Variables

A growing body of literature is now investigating modulatory factors that could affect functional connectivity in autism. A study looking at the effects of beta-blockers in an autism population measured functional connectivity in predefined ROIs (Narayanan et al. 2010) before and after administration of propranolol, nadolol, and placebo. They found that functional connectivity between the four ROIs (averaged for all ROI pairs) was higher for propranolol than placebo or nadolol. These findings highlight the importance of consideration of participants' medical regimens given the potential for asymmetric medication in autism and typically developing groups in other studies.

The relationship between functional connectivity in autism and genotype for the growing list of potential genetic susceptibility loci remains largely unknown. The first study directly investigating this relationship tested effects of genotype for locus contactin-associated protein-like 2 (CNTNAP2) was tested by Scott-Van Zeeland and colleagues (2010), who found increased functional connectivity for a medial prefrontal cortex seed to the posterior cingulate cortex (Default Mode Network) in the nonrisk group than for the risk group of the allele.

Although not directly measuring functional connectivity, resting-state BOLD low-frequency fluctuations were analyzed in a study by Lai and colleagues (2010) by examining the Hurst exponent of time series. The Hurst exponent is closely

related to fractal dimension, measuring temporal complexity of a time series. This study found greater randomness in the time series of autism participants across a wide range of brain regions.

11.14 Effects of Age

From childhood through adolescence, a series of changes have been described in normal development (Fair et al. 2007) that consist of a relative strengthening of long-range functional connectivity within coherent networks (integration) as well as increasing distinctness of different functional networks (segregation). These changes are diminished in autism throughout the prior studies reviewed, best illustrated by impaired functional connectivity within the default mode network and decreased negative correlations across a large set of brain regions (Anderson et al. 2011d).

The reduced effects of segregation and integration in autism were explicitly tested in a study by Rudie et al. that evaluated a cohort of adolescent autism and typically developing subjects during a task of emotional face processing. Task effects were regressed out, and functional connectivity was measured under a range of postprocessing strategies between the bilateral amygdala and right frontal operculum and the rest of the brain. Areas where the seeds were positively correlated tended to be more so in typically developing subjects, while areas that were negatively correlated were even more negatively correlated in control subjects (Rudie et al. 2012).

In a cohort of 41 typically developing and 39 autism participants, Wiggins and colleagues measured synchrony of resting-state BOLD images in the anterior and posterior hubs of the default mode network using a seed-independent self-organizing map technique (Wiggins et al. 2011). They found not only that anterior and posterior hubs were less correlated in autism but that this correlation increased less with age than for typically developing subjects. This also corresponds with findings of Anderson and colleagues that functional connectivity is most abnormal in children and adolescents with autism, with a trend toward normalization relative to typical development by about age 20 (Anderson et al. 2011d).

11.15 Methodological Considerations

Despite strong convergent findings across more than 50 primary reports, there are good reasons to maintain tentativity about findings of altered functional connectivity in autism. Over the time period these studies were performed, there has been considerable maturation of functional connectivity methods. One such example is evolving methods used for correction of nuisance signals such as heart rate and respiration. A common technique used in many but not all of the studies above is global signal regression, where the mean BOLD time series for a mask including the entire brain is regressed from each voxel's time series. This has been shown to generate spurious functional anticorrelations (Fox et al. 2009, Murphy et al. 2009). Although it is

possible that this technique could differentially affect patient groups, for example, if a network was spatially larger in one group compared to the other (Anderson et al. 2011b), this was not found to be the case in one study that tested functional connectivity with and without global regression (Rudie et al. 2012) (Table 11.1).

Most of the studies performed to date use data collected during a cognitive task, and it is possible that functional connectivity effects can be driven by task

Table 11.1 Overview of primary reports of functional connectivity in autism

Report	Sample	Task performed	Mean age	Brain regions	Effect of autism on connectivity
Cherkassky et al. (2006)	57 ASD 57 TD	Fixation	24	DMN	Decreased
Kennedy et al. (2006)	15 ASD 14 TD	Stroop	26	DMN	Decreased DMN Deactivation
Kennedy and Courchesne (2008b)	12 ASD 12 TD	Resting	27	DMN, ACN	ACN: no change DMN: decreased
Mason et al. (2008)	18 ASD 18 TD	Narrative comprehension	27	DMN	Decreased
Kana et al. (2009)	12 ASD 12 TD	Mental states of animated characters	24	DMN	Decreased
Monk et al. (2009)	12 ASD 12 TD	Resting	27	DMN	Anterior–posterior: decreased PCC–right hippocampal: increased
Weng et al. (2010)	16 ASD 15 TD	Resting	15	DMN	Decreased
Assaf et al. (2010)	16 ASD 16 TD	Resting	16	DMN	Decreased
Lombardo et al. (2010)	29 ASD 33 TD	Resting	27	DMN	Decreased
Welchew et al. (2005)	13 ASD 13 TD	Fearful faces	28	Medial Temporal	Decreased
Bird et al. (2006)	16 ASD 16 TD	Face/house discrimination	34	Occipital	Decreased attentional modulation of V1—extrastriate
Kleinhans et al. (2008)	19 ASD 21 TD	Face identification	24	Fusiform, limbic	Decreased
Monk et al. (2010)	12 ASD 12 TD	Emotional faces	26	Amygdala	Ant. temporal: decreased mPFC: increased
Villalobos et al. (2005)	8 ASD 8 TD	Visual button press	28	V1, left inferior frontal	Decreased
Brieber et al. (2010)	14 ASD 15 TD	Coherent motion	16	V5/MT	No difference
Damarla et al. (2010)	13 ASD 13 TD	Embedded figures	21	Visual, ACN	Decreased
Sahyoun et al. (2010)	12 ASD 12 TD	Pictorial reasoning	13	Visual, language	Decreased (DTI)
Keehn et al. (2012)	19 ASD 19 TD	Visual search	13	ACN, visual	Increased

(continued)

Table 11.1 (continued)

Report	Sample	Task performed	Mean age	Brain regions	Effect of autism on connectivity
Liu et al. (2011)	15 ASD 15 TD	Embedded figures	26	Frontal	Decreased
Koshino et al. (2005)	14 ASD 14 TD	n-back (letters)	28	ACN	Decreased
Koshino et al. (2008)	11 ASD 11 TD	n-back (faces)	27	ACN, fusiform	Decreased
Just et al. (2007)	18 ASD 18 TD	Tower of London	26	ACN	Decreased
Solomon et al. (2009)	22 ASD 23 TD	Response inhibition	16	ACN, occipital	Decreased
Agam et al. (2010)	11 ASD 14 TD	Saccade to target	27	ACN	Decreased
Kana et al. (2007)	12 ASD 12 TD	Go–no go	25	ACN	Decreased
Lee et al. (2009)	12 ASD 12 TD	Go–no go	11	ACN	Decreased (trend)
Di Martino et al. (2009)	25 TD	Resting	29	ACN	Decreased for high SRS scores
Ebisch et al. (2011)	14 ASD 15 TD	Resting	16	Insula	Decreased Ant. insula to post. insula
Just et al. (2004)	17 ASD 17 TD	Sentence comprehension	N/A	Language	Decreased
Kana et al. (2006)	12 ASD 12 TD	Sentence comprehension	21	Language, parietal	Decreased (trend)
Noonan et al. (2009)	10 ASD 10 TD	Visual word recognition (memory)	24	Left occipital, left middle frontal, left parietal	Increased connectivity to seeds (medial frontal, posterior temporal)
Jones et al. (2010)	17 ASD 20 TD	Verbal fluency	17	Language	Decreased
Mizuno et al. (2011)	15 ASD 15 TD	Linguistic perspective ("I" vs. "you")	25	Anterior insula, precuneus	Decreased
Lai et al. (2012)	39 ASD 21 TD	Song, speech	10	Language	No group comparison (autism sedated)
Dinstein et al. (2011)	29 ASD 13 Lang. delay 30 TD	Natural sleep with auditory stimuli regressed out	2	Language	Decreased interhemispheric correlation (inf. frontal, sup. temporal)
Mostofsky et al. (2009)	13 ASD 13 TD	Finger tapping	10	Motor	Decreased
Mizuno et al. (2006)	8 ASD 8 TD	Visuomotor	28	Thalamus, cortex	Mostly increased
Turner et al. (2006)	8 ASD 8 TD	Visuomotor	28	Caudate, cortex	Left caudate: increased Right caudate: decreased
Di Martino et al. (2011)	20 ASD 20 TD	Resting	11	Caudate, putamen, cortex	Mostly increased
Paakki et al. (2010)	27 ASD 27 TD	Resting	15	Whole brain (ReHo)	Right STS, inf. frontal, insula, postcentral: decreased Right thalamus, left inf. frontal: increased

(continued)

Table 11.1 (continued)

Report	Sample	Task performed	Mean age	Brain regions	Effect of autism on connectivity
Shukla et al. (2010)	26 ASD 29 TD	Visual search (regressed out)	14	Whole brain (ReHo)	Superior parietal, ant. prefrontal: decreased Right temporal: increased
Wicker et al. (2008)	12 ASD 14 TD	Judgment of facial affect (structural equation modeling)	25	Amygdala, fusiform, DLPFC, DMPFC, VLPFC, V1, STS	Mostly decreased, especially top–down frontal to temporal/limbic regions
Shih et al. (2010)	14 ASD 14 TD	Semantic decision or letter detection	24	Right inferior frontal, STS, inferior parietal	Increased between three seeds and anterior cingulate/prefrontal
Schipul et al. (2012)	18 ASD 18 TD	Social judgment learning	22	25 ROIs	Decreased in autism, with smaller increase with learning
Von dem Hagen et al. (2012)	18 ASD 25 TD	Resting	28	DMN, amygdala, frontal insula, anterior cingulate	Decreased DMN connectivity Decreased connectivity between amygdala and insula
Anderson et al. (2011a)	53 ASD 39 TD	Resting	21	Homotopic	Decreased interhemispheric
Anderson et al. (2011a)	48 ASD 53 TD	Resting	22	Whole brain	Decreased positive correlations; increased negative correlations
Narayanan et al. (2010)	10 ASD	Phonological decision making	24	Left inferior frontal, fusiform, parietal, temporal	Increased connectivity with propranolol
Scott-Van Zeeland et al. (2010)	16 ASD 16 TD (9 risk, 23 nonrisk allele); Replication 39 TD	Implicit learning task	13	Medial prefrontal, posterior cingulate	Decreased in risk allele group
Rudie et al. (2012)	23 ASD 25 TD	Emotional face processing	13	Amygdala, occipital, prefrontal, inferior frontal	Right operculum, bilateral amygdala: decreased Right operculum to frontal: increased
Wiggins et al. (2011)	39 ASD 41 TD	Resting	15	DMN	Decreased
Lai et al. (2010)	30 ASD 33 TD	Resting	27	Whole brain (complexity)	Greater randomness in autism
Gotts et al. (2012)	31 ASD 29 TD	Resting	17	Whole brain	Decreased in social brain regions

performance that is asymmetric between groups. For example, if an autism population exhibits less activation of the fusiform face area during a facial processing task, then it will be synchronously active during the task with other task-engaged areas to a lesser extent than a control population. Studies using resting-state acquisitions may avoid stimulus-driven changes in connectivity, but it is important to note that the "resting state" is simply another task, albeit less constrained. Subjects can perform vastly different cognitive tasks during the resting state that may have little basis in structural connectivity in the brain. Research continues to evolve on the reliability and biological interpretation of functional connectivity differences.

Likely more significant are the effects of small amounts of uncorrected motion that might have persisted in datasets differentially between patient populations. Such small amounts of motion have been shown to significantly lessen effect sizes of functional connectivity differences during development such as segregation and integration (Power et al. 2012), and these changes are similar to the main effects seen in autism: decreased long-range connectivity. Moreover, correction methods such as despiking or individual frame removal (Power et al. 2012; Van Dijk et al. 2012) were employed in almost none of the studies reviewed. One of the only studies to use more recent techniques for rigorous motion correction was one in which increased connectivity was found, albeit in a domain where autism subjects do not typically show behavioral impairments (Keehn et al. 2012).

Sample sizes have also been small across most of the published studies. Functional connectivity measurements have a large amount of inherent noise, requiring long imaging times or large number of subjects to converge to precise estimates (Anderson et al. 2011a, Ferguson and Anderson 2011). Given the need for characterizing differences in functional connectivity across brain regions, subject age, and other covariates, the multiple comparison problem becomes extreme, and full characterization of connectivity disturbances in autism will likely require much larger pools of data than have been previously used. Fortunately, there have been recent efforts to organize data sharing among laboratories to allow combined datasets with much greater power. The Autism Brain Imaging Data Exchange (http://www.childmind.org/en/healthy-brain-network/abide) has attracted 15 contributing sites, with resting-state data from over 1,000 subjects, with public release of data anticipated for fall 2012. A separate data-sharing initiative, the National Database for Autism Research (http://ndar.nih.gov), has also begun collection of publicly available fMRI data to allow multisite analyses.

Finally, most of the studies performed have characterized functional connectivity in adults. Yet studies that have included children and adolescents have found that connectivity differences tend to be greater in younger subjects, with normalization to the typically developing population beginning around age 20 (Anderson et al. 2011d, Wiggins et al. 2011). Studies in young children are difficult because subjects in the first decade of life are difficult to scan without sleep or sedation and such scans can dramatically affect functional connectivity. Nevertheless, continued exploration of functional connectivity in younger patients is important to characterize the evolution of brain development in autism if diagnosis or prognosis is to be attempted in a clinically relevant time frame by MRI.

11.16 Open Questions

With a broad overview of published results, several important open questions arise.

- At what age do functional connectivity abnormalities in autism develop?
- Can functional connectivity be used to characterize the heterogeneity of the autism population by subtyping, predicting prognosis, or establishing endophenotypes?
- What treatment strategies can be constrained or tested by our knowledge of abnormal connectivity in the autism brain?
- How do observed functional connectivity patterns affect behavior, stability of distributed brain networks, and cognitive performance?
- Are functional connectivity patterns genetically determined or the product of experience and training, and can these patterns be modified by interventions?
- What is the relationship between excitatory and inhibitory neural connections and resulting functional connectivity in autism?
- At what spatial distances in the brain is "local overconnectivity" present, if at all?
- What abnormalities are present in between-network functional connectivity in autism?

11.17 Conclusion

Studies of functional connectivity in autism show variable support for a generalized underconnectivity hypothesis in autism. The majority of published studies show deficits in inter-regional brain synchronization involving the default mode network, homotopic left–right connections, and social brain regions that correspond to many of the clinical deficits seen in autism patients. As with the larger functional MRI task-based literature, deficits are most apparent in neural systems associated with impaired function in autism. Connections between networks also show abnormalities, particularly between the default mode and attention control networks. Notable exceptions where overconnectivity in autism has also been reported include the corticostriatal network. Future work will help establish whether these findings may yet be merged into a consistent theory of connectivity anomalies that take into account the spatial distribution of connectivity abnormalities in a heterogeneous population by integrating data from multisite imaging data-sharing initiatives with the power to provide definitive answers to these questions.

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Biography

Jeffrey S. Anderson received his M.D. and Ph.D. degrees from Northwestern University in neuroscience. He completed residency training in diagnostic radiology and a neuroradiology fellowship before joining the faculty at the University of Utah School of Medicine. He directs the fMRI neurosurgical mapping service, and is principal investigator for the Utah Functional Neuroimaging Laboratory. Dr. Anderson's lab studies brain networks using functional imaging techniques, with particular interest in autism.

Chapter 12
EEG Analyses in the Assessment of Autistic Disorders

Robert Coben, Robert J. Chabot, and Laurence Hirshberg

12.1 Introduction

Autistic spectrum disorders (ASD) are a heterogeneous group of pervasive developmental disorders including autistic disorder, Rett's disorder, childhood disintegrative disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), and Asperger's disorder. Children with ASD demonstrate impairment in social interaction, verbal and nonverbal communication, and behaviors or interests (DSM-IV-TR; APA 2000). ASD may be comorbid with sensory integration difficulties, mental retardation, or seizure disorders. Children with ASD may have severe sensitivity to sounds, textures, tastes, and smells. Cognitive deficits are often associated with impaired communication skills. Repetitive stereotyped behaviors, perseveration, and obsessionality, common in ASD, are associated with executive deficits. Executive dysfunction in inhibitory control and set shifting have been attributed to ASD (Schmitz et al. 2006). Seizure disorders may occur in one out of four children with ASD, frequently beginning in early childhood or adolescence.

Autistic disorder includes the following triad of symptoms (1) impaired social interaction, failure to develop peer relationships, or lack of initiating spontaneous activities; (2) deficits in communication including delay in or lack of spoken language, inability to initiate or sustain conversation with others, stereotyped

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repetitive use of language or idiosyncratic language; and (3) restricted repetitive and stereotyped behavior, interests, inflexible adherence to routines or rituals, and repetitive motor patterns (e.g., hand or finger flapping or twisting) (DSM-IV-TR; APA 2000).

Individuals with Asperger's disorder frequently have high levels of cognitive functioning, engage in literal pedantic speech, experience difficulty comprehending implied meaning, exhibit problems with fluid movement, and manifest inappropriate social interactions. Pervasive developmental disorder-not otherwise specified (PDD-NOS) reflects deficits in language and social skills, which do not meet the criteria of other disorders. In contrast, persons with childhood disintegrative disorder and Rett's disorder both have normal periods of early development followed by loss of previously acquired skills. Common features among all these conditions include communication and social skill deficits. There is considerable variability in terms of onset and severity of symptomatology within the autistic spectrum of disorders (Attwood 1998; Hamilton 2000; McCandless 2005; Sicile-Kira 2004).

Research reviewing the epidemiology of autism (Center for Disease Control and Prevention; CDC 2009) reported between 1 in 80 and 1 in 240 children in the United States diagnosed with the disorder. A report of just 3 years ago (Center for Disease Control and Prevention 2009) suggested a prevalence of 1 in 110 and as high as 1 in 70 boys. In their most recent report, the CDC (2012) suggests that the rate has risen to 1 in 88. ASDs are five times more likely in boys for which it is seen in 1 out of 54 male children. According to Blaxill (2004), the rates of ASD were reported to be <3 per 10,000 children in the 1970s and rose to >30 per 10,000 in the 1990s. This rise in the rate of ASD constituted a tenfold increase over a 20-year interval in the United States. These findings make accurate assessment of autistic individuals and their underlying neurophysiology a priority.

12.2 EEG Assessment in Autism

Multiple neuroimaging studies have demonstrated brain anomalies in autistics compared to healthy controls (McAlonan et al. 2004; Page et al. 2006). The electroencephalography (EEG) was one of the earliest techniques used to investigate the neurobiology of autism (Minshew 1991). The recognition of a high instance of EEG abnormalities and of seizure disorders in the autistic population was among the earliest evidence of a biologic basis for disorder (Minshew 1991). Moreover, the EEG is a premiere tool to assess neural dysfunctions related to autism and seizures due to its' noninvasive nature, availability, and utility in detailing these types of difficulties.

Consistent with this, seizures and epilepsy have been commonly observed in autistic samples. Recent analyses have estimated the prevalence of seizure disorders in autistic series at anywhere from 20 to 46 %. Based on recent analyses, the prevalence of seizure disorders in autistic series is estimated at about 36 % (Danielsson et al. 2005; Hara 2007; Hughes and Melyn 2005; Parmeggiani et al. 2007). In fact, it has been reported that the autistic population has about 3–22-fold increased risk

of developing seizure disorders as compared to the normal population (Volkmar and Nelson 1989). Increasing cognitive/intellectual disability appears to be associated with seizure disorders in Autism. Subclinical seizure activity or paroxysmal discharges occur in an even higher proportion of autistics, but the significance of these remains uncertain (Hughes and Melyn 2005; Parmeggiani et al. 2007). Ray et al. (2007) have suggested that the initial phase of cortical spikes may relate to underlying intracranial foci. Other work has suggested that EEG spikes may reflect underlying morphological brain abnormalities (Shelley et al. 2008) and/or metabolic disturbances (Kobayashi et al. 2006).

Recent estimates suggest that approximately one-third of all autistic children experience a regression in speech or behavior early in life (Canitano 2007). Tuchman and Rapin (1997) were unable to relate early regression to seizure disorders but suggested that the EEG is abnormal in a greater proportion of autistic children that regress than those that do not. Abnormal electroencephalogram (EEG) recordings are also present in the majority of autistic children with seizure disorders (Hughes and Melyn 2005). In a more recent study, Parmeggiani et al. (2010) demonstrated that in a large inpatient sample, 58 % of adults with autism aged 20 or older had experienced epilepsy or a seizure during their lifetime.

For these reasons, experts in the field have recommended the use of routine and sleep EEGs in the evaluation of autistic disorders, especially when there has been regression or there are signs of possible seizures. In fact, seizure detection has been the primary role of the EEG for decades. Neurologists and epileptologists routinely use visual inspection of data as a basis for diagnosing seizure-related disorders.

When EEG assessment is processed and analyzed with the most advanced techniques, it can be invaluable for screening for possible seizures, evaluation of autistic disorders, and assessing the neurophysiological challenges of children with ASD. Presently, assessment of regional brain dysfunction usually requires functional brain imaging techniques as static measures tend to find few abnormalities in autistic disorders. This would include techniques such as functional MRI, PET, single-photon emission computed tomography, magnetoencephalography (MEG), and even EEG. Some of these techniques require sedation or injection of radioactive material so as to make participation difficult for a typical autistic child. EEG however appears to be the most clinically available and again least invasive of these techniques. Further, it has been found that unique patterns of regional dysfunction could be discerned through the quantitative analysis of the EEG.

12.3 Quantitative EEG Findings and ASD

A review of the existing literature identified 14 studies that used quantitative techniques to analyze differences in EEG (QEEG) activity between children with autistic spectrum disorder (ASD) and normal controls with conflicting results. Two studies showed decreased delta frontally (Coben et al. 2008; Dawson et al. 1982), while one found increased activity in the delta frequency range (Murias et al. 2007).

Two studies reported increased generalized delta or described "slowing" (Cantor et al. 1986; Stroganova et al. 2007). Two studies showed theta increases (Coben et al. 2008; Small et al. 1975), while one study reported reduced theta (Dawson et al. 1982). By contrast, findings have been quite consistent within the alpha through gamma frequency range. All studies reported reduced alpha power (Dawson et al. 1982; Cantor et al. 1986) and increased beta (Coben et al. 2008; Chan and Leung 2006; Rossi et al. 1995) and gamma power (Orekhova et al. 2006). Multiple studies report a lack of hemispheric differences in QEEG spectral power in autistic samples compared to findings of hemispheric differences in normal controls. Autistic children showed decreased power asymmetry when compared to normal or mentally handicapped controls (Dawson et al. 1982; Ogawa et al. 1982). Three studies investigated cortical connectivity in ASD samples using QEEG coherence measures, with all reporting reduced connectivity, especially over longer distances (Cantor et al. 1986; Coben et al. 2008; Lazarev et al. 2004).

Sample sizes by and large have not been large enough to allow for investigation of the observed inconsistencies in findings reported above. One possibility is that the specific QEEG abnormalities found may be associated with differences in functioning and are characteristic of the phenotypic heterogeneity of autism spectrum disorder. Such an analysis has been useful in QEEG research in attention deficit disorder (Chabot et al. 1999, 2005; Clarke et al. 2003a, b). The present study was designed to document QEEG differences between a large sample of children diagnosed with autistic spectrum disorder and a matched sample of children with no known neurological or psychiatric disorders. The goal was to document the specific types of QEEG profiles found within this population, to develop a QEEG feature-based discriminant function (possible biomarker) to distinguish children with autistic spectrum disorder from the normal population of children, and to use the QEEG source localization technique VARETA to delineate the neuroanatomical structures that are dysfunctional in ASD.

12.3.1 Methods and Material

Clinical Population: All children were referred to the Neurorehabilitation and Neuropsychological Center in Massapequa, New York. A sample of 91 children was entered into this study (mean age = 9.9 years; sd = 3.4). None of the children were receiving psychotropic medication at the time of testing. Children with histories of drug abuse, head injury, or other neurological disorders were excluded. IRB approval was obtained for this study and all data were de-identified prior to processing. All participants in this study met diagnostic criteria for either autistic disorder, Asperger's disorder, childhood disintegrative disorder, or pervasive developmental disorder-not otherwise specified as described by the DSM-IV. All diagnoses were made by the first author, a licensed clinical psychologist and practicing neuropsychologist, and were based upon patient and parent interviews supplemented by

scores on the Gilliam Asperger's Disorder Scale and the Gilliam Autism Rating Scale (Gilliam 1995, 2001).

Normal Population: The normal controls included 91 children between the ages of 6 and 17 matched to the ASD population based upon age and gender. All normal subjects were free of neurological or medical disease; had no history of head injury, drug, or alcohol abuse; were of normal IQ; showed evidence of adequate functioning at home/school for the past 2 years; and had not taken any prescription medication for at least 90 days prior to evaluation.

Quantitative EEG Methodology: The Neurometric method of EEG data collection and analysis was utilized (John et al. 1977, 1988). Patients were seated comfortably in a sound and light attenuated room during the evaluation. Recording electrodes were placed over the 19 standard regions defined by the International 10/20 system referenced to linked ears. All electrode impedance levels were kept below 5,000 Ω. A differential recording channel above and below the right eye was used to monitor eye movement artifact. Twenty to thirty minutes of continuous eyes-closed resting EEG was recorded from all children. An experienced EEG technician selected the first 1–2 min of artifact-free EEG from this record for analysis. Particular care was taken to prevent EEG contamination due to drowsiness and to exclude EEG segments contaminated by artifact.

The artifact-free EEG from each channel was converted from the time to the frequency domain via fast Fourier transform (FFT). Two QEEG measures related to the frequency distribution of the EEG were calculated: absolute power defined as the amount of energy in the delta (1.5–3.5 Hz), theta (3.5–7.5 Hz), alpha (7.5–12.5 Hz), and beta (12.5–25.0 Hz) frequency bands as well as the total power across all frequency bands (1.5–25.0 Hz); relative power in each frequency band defined as the amount of energy in each band divided by total power. Two QEEG measures were calculated that measured EEG connectivity across cortical regions including power asymmetry in each frequency band between the left and right hemispheres and waveform coherence or the correlation of EEG across selected cortical regions. Each measure was mathematically transformed to conform to a normal distribution and age-regressed and compared to the mean and standard deviation of that measure obtained from a previously published normal database of 310 children using a z or standard score. Conversion to z-scores allows each QEEG measure to be described using a common metric related to the probability of coming from the normal population, and as such, these features can be used together in subsequent discriminant analyses.

Variable resolution electromagnetic tomography (VARETA) is a three-dimensional source localization method that uses surface-recorded EEG to identify the most probable neuroanatomical generators of each EEG frequency band and maps these results onto a probabilistic brain atlas resembling slices obtained from an MRI (Bosch-Bayard et al. 2001). When z-score transformed relative to a normal population, these VARETA brain images can be used to depict the cortical and subcortical structures involved in the pathophysiology of various neurocognitive disorders (di Michele et al. 2005).

12.3.2 Results

12.3.2.1 QEEG Absolute Power Findings

Separate multivariate analyses of variance were calculated comparing the normal children and those with ASD for the delta, theta, alpha, and beta frequency bands across the 19 recording regions. For delta absolute power, all 19 univariate *F*-ratios reached the $p < 0.0001$ level of significance (d$f = 1,180$) with the overall multivariate *F*-ratio = 23.6 ($p < 0.0001$, d$f = 19,162$). ASD children showed a generalized deficit of delta absolute power that was greater in frontal and central than in more lateral and posterior regions. Theta absolute power was increased in the ASD population with univariate *F*-ratios significant at $p < 0.001$ in frontal, central, and left parietal and posterior/temporal regions (Multi $F = 10.9$, $p < 0.0001$). While the overall multivariate *F*-ratios for alpha and beta absolute power were significant ($F = 8.6$, $p < 0.001$; and $F = 7.1$, $p < 0.0001$), none of the univariate *F* values reached the $p < 0.001$ level of significance.

12.3.2.2 QEEG Relative Power Findings

For delta relative power, all 19 univariate *F*-ratios reached the $p < 0.0001$ level of significance with the overall multivariate *F*-ratio =16.6 ($p < 0.0001$). ASD children showed a generalized deficit of delta relative power that was greater in frontal and central than in more lateral and posterior regions. Theta relative power was increased in the ASD population with the univariate *F*-ratios significant at $p < 0.001$ for frontal and anterior and posterior/temporal regions (Multivariate $F = 4.4$, $p < 0.0001$). Alpha relative power was increased in the ASD population in all regions ($p < 0.001$) except for posterior/temporal and occipital recordings (Multivariate $F = 7.6$, $p < 0.0001$). Beta relative power was increased in ASD for all regions ($p < 0.001$) except for the anterior temporal and occipital regions (Multivariate $F = 6.3$, $p < 0.0001$).

12.3.2.3 QEEG Power Asymmetry Findings

Multivariate *F* values reached significance for each frequency band (*F* between 3.3 and 5.9 with *p* between 0.002 and 0.0001; d$f = 8,173$). Univariate *F*-ratios reached the $p < 0.001$ level of significance for frontal/lateral and parietal delta and theta, for frontal/lateral alpha, and for parietal beta. In frontal/lateral and parietal comparisons, ASD children had increased right hemisphere delta and theta and decreased alpha power relative to left hemisphere power values when compared to the normal population. In parietal comparisons, ASD children had increased left hemisphere beta power relative to right hemisphere power values when compared to the normal population.

12.3.2.4 QEEG Coherence Findings

Multivariate F values reached significance for each frequency band (F between 2.9 and 4.8 with p between 0.004 and 0.0001). Univariate F-ratios reached the $p < 0.001$ levels for frontal/lateral theta, alpha, and beta; for anterior and posterior/temporal alpha; and for posterior/temporal beta coherence. Frontal/lateral coherence values were increased (hypercoherent) in ASD in comparison with the normal population. Anterior and posterior/temporal coherence was decreased (incoherent) in ASD in comparison with the normal population.

12.3.2.5 QEEG Heterogeneity in ASD

A combination of factor and cluster analysis procedures was utilized to determine whether or not the relative power QEEG frequency distribution could be used to delineate subtypes of ASD. Factor analysis was used to reduce the number of relative power variables (19 regions by four frequency bands) that could be entered into cluster analysis in keeping with maintaining a minimum of 10/1 subject to variable ratio. Factor analysis with varimax rotation was performed for each frequency band across 19 monopolar regions. For each analysis, three factors were obtained. These factors accounted for 81.9 % of the variance for delta, 85.5 % for theta, 89.8 % for alpha, and 85.2 % for beta relative power. An examination of the rotated factor loadings revealed factors which corresponded to frontal plus anterior temporal, parietal/occipital plus posterior/temporal, and central regions for delta, theta, and alpha relative power. For beta relative power, there were frontal/central, parietal/occipital plus posterior/temporal, and anterior temporal factors. None of the factors was related to left versus right hemispheric differences.

Cluster analysis was then performed using mean relative power values that were averaged across the three regions identified by the factor results just described. Thus, there were 12 QEEG measures (four frequency levels by three average factor regions) entered into the cluster analysis of the 91 ASD children. Four, five, and six cluster solutions were examined. For all solutions, each cluster had a deficit of delta relative power. For the four cluster solution, there were a theta excess, alpha excess, theta and alpha excess, and beta excess cluster. With five clusters, the beta cluster was divided into two clusters, one with slight theta excess and the other with slight alpha excess. With six clusters an additional small theta excess cluster with elevated alpha and beta was seen. Figure 12.1 presents the relative power average z-score head maps for the individuals within each of the clusters in the five cluster solution.

12.3.2.6 QEEG Discriminant Analysis Findings

A step-wise discriminant analysis was calculated comparing the normal children with the children with autistic spectrum disorder. QEEG variables entered were selected using analysis of variance comparisons between the two groups with those

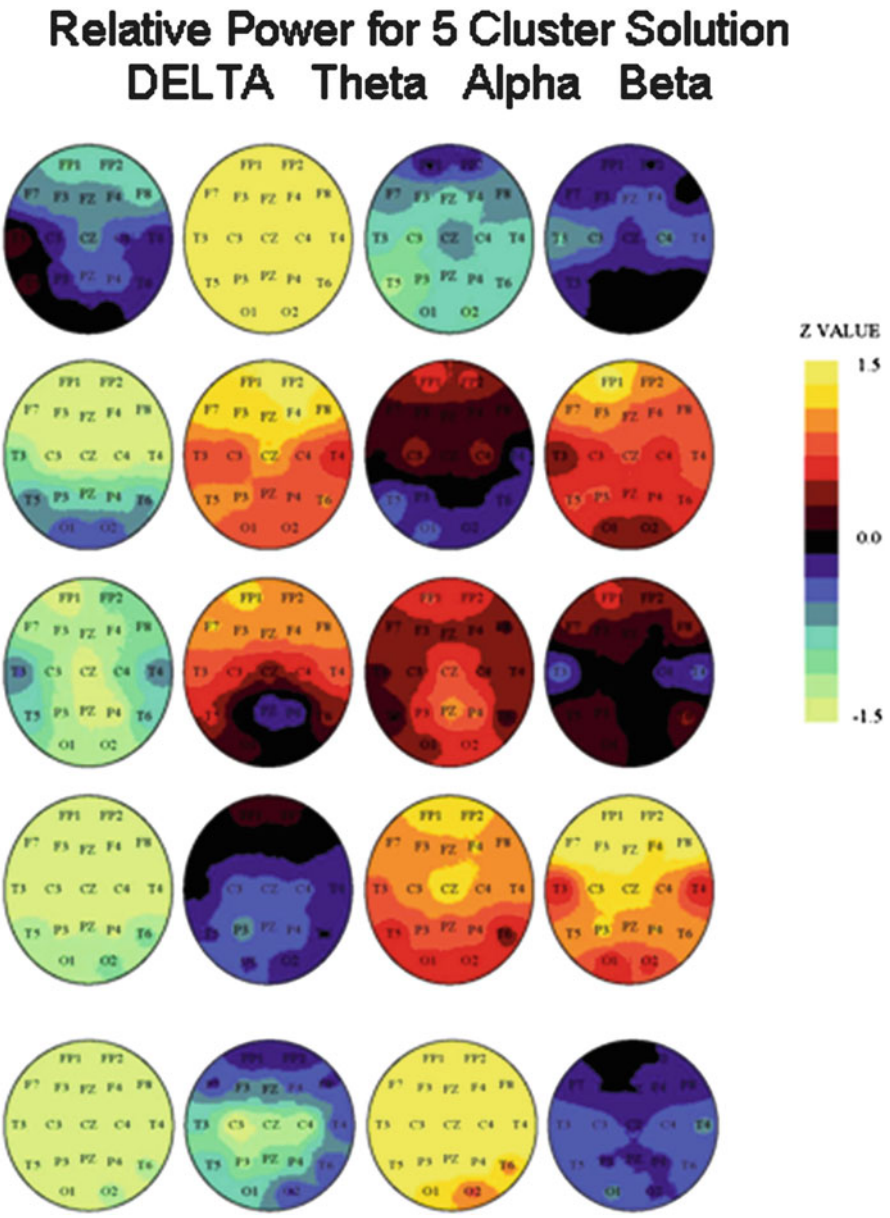

Fig. 12.1 Group average QEEG Z images for five QEEG subtypes of ASD. Images shown are oriented with nose up, left on left, and are color coded in standard deviation units (*z*-scores), with excesses in *red/yellow* and deficits in *blue/green*. In group average images, the significance of the *z*-score is estimated multiplying the square root of the group size by the *z*-value; thus, in these images, the extremes of the scale are $p < 0.001$

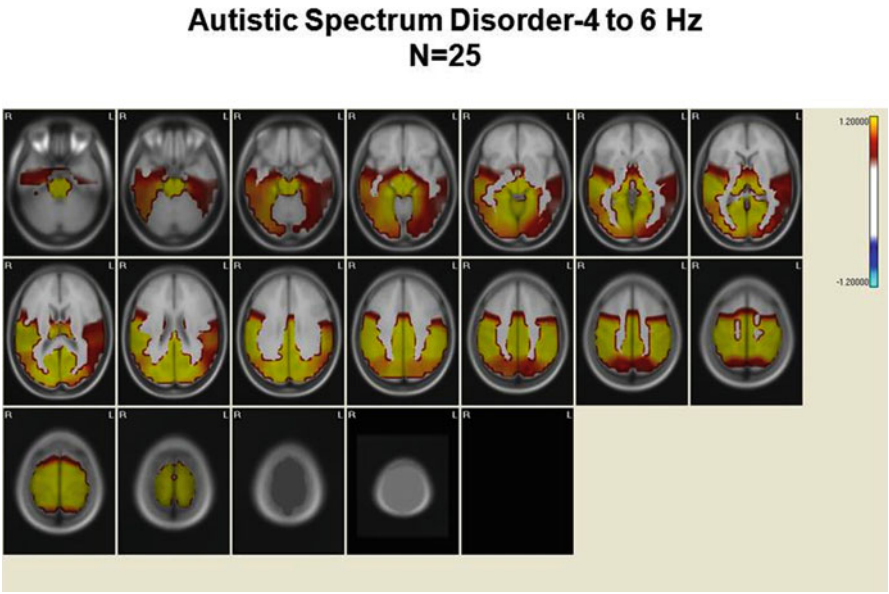

Fig. 12.2 The *panels* depict trans-axial images of the sources identified using VARETA, showing the mathematically most probable generators of the most abnormal QEEG activity for the group, and represent the mean *z*-values of each voxel computed across the group of ASD children and color-coded for significance. These images follow the radiological convention, with the right side of the head depicted on the left side of the slice

variables with the highest *F*-ratios and lowest intercorrelations chosen. A total of five variables were utilized resulting in 92.3 % (sensitivity) correct identification of normal children and 95.6 % (specificity) correct identification of ASD children. A jack-knife replication resulted in 92.3 % correct identification of normal and 95.6 % correct identification of ASD children. The positive predictive value was 95.5 % and the negative predictive value was 92.6 %. QEEG variables utilized included central delta mean frequency, central theta coherence, central delta relative power, right frontal/temporal alpha asymmetry, and right frontal/temporal theta relative power.

12.3.2.7 VARETA EEG Source Localization Findings

Group average VARETA images were constructed for each of the five above defined subtypes separately for the ASD children. An examination of these images revealed consistent findings across QEEG subtypes. Figure 12.2 presents a group average VARETA image in the axial plane for the theta excess subtype. The VARETA image uses threshold scaling such that colors shown represent statistically significant deviations from the normal population. ASD children were characterized by increased theta activity in the cerebellum, thalamus, hippocampus, parahippocampal, cuneus,

cingulate, and lingual gyrus and in temporal, precentral, postcentral, parietal, and occipital cortical regions. Note that the anatomical location of abnormal neurophysiological activity was very consistent across ASD subtypes and is illustrated in Fig. 12.2. Consistent differences were seen between the ASD subtypes and normal children at each frequency, and these differences showed virtually the same pattern of anatomical abnormality across subtypes. In other words, despite the different frequency distributions noted between ASD subtypes, the neuroanatomical structures identified by VARETA as showing abnormal activity are consistent and may represent the neuroanatomical structures which show dysfunction in ASD.

12.4 Discussion

In the largest study of its kind, differences between the ASD and normal populations were seen in both absolute and relative power for the delta, theta, alpha, and beta frequency bands. One of the more intriguing findings is that of a prominent delta absolute and relative power deficit in the autistic children. While this delta deficit was generalized, it was most prominent in frontal and central regions. Coutin-Churchman et al. have shown delta deficits to be associated with cerebral atrophy (Coutin-Churchman et al. 2003). Frontal white matter volume has also been negatively correlated with frontal delta power (Babiloni et al. 2006). Delta activity is believed to originate in deep cortical neurons and in the thalamus (John and Prichep 2006). Evans (2003) postulated a thalamocortical network responsible for the integration of brain electrical activity and that deficient delta activity may signify a weakness in this system. Consistent with this model have been the animal studies demonstrating associations between deficient slow-wave activity and functioning of the striatal dopaminergic system (Kitaoka et al. 2007; Alper 1999; Binienda et al. 2002). This theory is interesting as it relates to autistics given the pervasiveness of their neural connectivity impairments (Coben and Myers 2008b; Minshew and Williams 2007).

Excess relative and absolute theta power were more localized occurring mainly in frontal, central, and temporal regions. This finding is supported in the literature where three (Coben et al. 2008; Small et al. 1975; Chan and Leung 2006) of four previous studies have reported theta excesses in ASD, while only one (Dawson et al. 1982) has shown a theta deficit. Our finding of an alpha excess in all but posterior cortical regions differs from that reported in the literature (Dawson et al. 1982; Cantor et al. 1986), although the finding of increased beta especially in frontal and central regions is consistent with the previous literature (Coben et al. 2008; Chan and Leung 2006; Rossi et al. 1995). This inconsistent finding for relative and absolute alpha power may be explained by examining the cluster analysis results that show the heterogeneity of ASD relative power findings. Note that four of five clusters are characterized by some degree of alpha excess, although cluster 1 is characterized by an alpha deficit. An examination of the cluster analysis findings also reveals that 5/5 clusters showed some degree of delta deficit, three of five showed theta excess and 1/5 theta deficit, and 2/5 some degree of beta excess.

Differences between the normal and ASD populations were found in the power relationships between the two hemispheres (power asymmetry). The children with ASD showed increased right hemisphere delta and theta and decreased alpha power relative to their left hemisphere when compared to normal children's power relationships with this difference present in lateral frontal and parietal regions. On the other hand, ASD children had increased left hemisphere beta power relative to their right hemisphere when compared to normal children's hemispheric power relationships. Further, our findings indicate that ASD is characterized by increased frontal coherence and decreased anterior and posterior/temporal coherence between the two cerebral hemispheres. These findings suggest that the brain dysfunction in autistic disorders is often bilateral and impacts both anterior and posterior axes. Alternatively, one could view the brain dysfunction in autism as an abnormality in connectivity that disrupts function in multiple regions (Minshew and Williams 2007). This would suggest that such connectivity impairments are prevalent in autistic children. This is consistent with the findings of Coben et al. (2008). Such an interpretation is also supported by the literature suggesting that autism is primarily a disorder of neural connectivity.

12.4.1 Autism as a Disorder of Neural Connectivity

There is increasing evidence that the cardinal disruptions in autism are represented by disruptions in brain connectivity (Courchesne and Pierce 2005; Mak-Fan et al. 2012; Minshew and Williams 2007). There is mounting evidence of head enlargement as a result of brain overgrowth early in life (first 1–2 years) (Courchesne et al. 2001, 2003) as a result of enhancements in frontal white matter and minicolumn pathology (Carper and Courchesne 2005; Casanova et al. 2002; Herbert et al. 2004; Vargas et al. 2005). This overgrowth, then, leads frontal over-connectivity (Coben and Myers, 2008b; Courchesne and Pierce 2005; Rinaldi et al. 2008) which interferes with the normal developmental trajectory. This disruption, theoretically, then halts the natural developmental progression in which anterior to posterior brain regions would enhance their synchronization and specialization of functions (DaMasio 1989; Supekar et al. 2009). This pattern, in fact, was observed in our data above showing frontal hypercoherence and bilateral temporal hypocoherences.

Other data support this hypothesis as well. For example, Mak-Fan et al. (2012) examined changes in diffusivity with age within frontal, long-distance, longitudinal, and interhemispheric tracts across ages 6–14. Their findings showed that while typically developing controls change and evolve on such measures, children with autism did not. This suggests that such connectivity difficulty exists and persists in such children. More specifically, frontal and local (short neuronal paths) hyperconnectivity has been shown to be present in autistic samples (Li et al. 2012; Wass 2011). In addition, there is other recent data showing hypoconnectivity in long-distance and posterior to anterior or temporal regions in autistics. Isler et al. (2010) have shown low interhemispheric coherence in visual evoked potentials in such children.

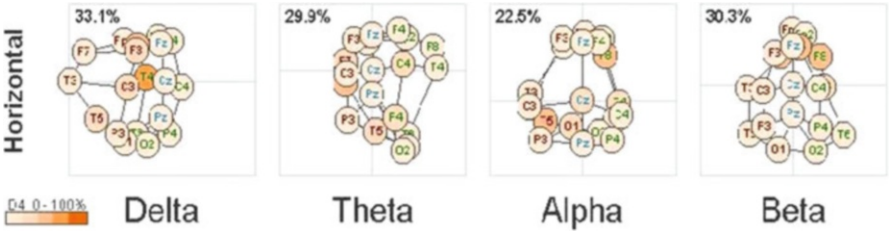

Fig. 12.3 NeuroRep multivariate connectivity analyses showing eigen images in the horizontal place across delta, theta, alpha, and beta frequencies. Observable features include (1) frontal hyperconnectivity in the alpha and beta frequency bands, (2) left anterior temporal/posterior frontal to posterior/temporal–parietal hypocoherence in the delta band, (3) right medial temporal to occipital–parietal–posterior/temporal hypocoherence in the theta band, and (4) bilateral frontal/temporal hypocoherences in the alpha band

Studies of functional connectivity related to visuospatial processing and the social-emotional processing networks have also shown reduced connectivity compared to healthy controls (Ameis et al. 2011; McGrath et al. 2012; vondem Hagen et al. 2012). Similarly, low functional connectivity has been shown to be related to poor language processing in autistic children (Kana et al. 2006). Many of these studies used three-dimensional imaging techniques such as MRI, fMRI, or DTI (diffusion tensor imaging).

Interestingly, EEG/QEEG studies of coherence have shown similar findings. In our study reviewed above, frontal hypercoherence and bilateral posterior-temporal hypocoherences were found. Similarly, high frontal coherence has been observed in other studies (Coben and Padolsky 2007; Murias et al. 2007). In addition, EEG technology has been able to demonstrate long-range, anterior to posterior and temporal hypocoherences (Coben et al. 2008; Murias et al. 2007). It has further been observed that multivariate strategies to assess coherence metrics are more accurate and effective than their pairwise counterparts (Barry et al. 2005; Kus et al. 2004; Pollonini et al. 2010). A clinical example is presented below in Fig. 12.3. This is taken from the first author's clinical practice and represents the EEG multivariate coherence analysis on a child with autism. This system of coherence assessment was created by Hudspeth (Multivariate connectivity within a spherical brain space. Unpublished manuscript, 2009) and is contained within the NeuroRep QEEG Software system. This system uses pairwise coherence data and through a principal components, statistical analysis converts the data into three-dimensional multivariate coherence eigen images. These eigen images can be viewed as an image in three-dimensional space representing the functional proximity or coherence among the various electrodes based on the 10/20 International EEG recording system (Niedermeyer and Lopes da Silva 2004). As such, electrode positions that are closer in proximity reflect greater hypercoherence, and electrodes that are further apparent are indicative of greater hypocoherences. In this case example, it is clear that this patient manifests simultaneous overlapping neurophysiological disruptions (as is observed in all autistic children). These include (1) frontal hyperconnectivity in the alpha and beta frequency bands, (2) left anterior

temporal/posterior frontal to posterior/temporal–parietal hypocoherence in the delta band, (3) right medial temporal to occipital–parietal–posterior/temporal hypocoherence in the theta band, and (4) bilateral frontal/temporal hypocoherences in the alpha band. These data can then be used for assessment purposes and treatment planning. The use of neurofeedback with these metrics as a basis has been shown to be more effective than other types of neurofeedback for children on the autistic spectrum (Coben and Myers 2008a).

12.4.2 EEG as a Discriminant in Autism

There has been great interest in techniques that can diagnose autism and younger and younger ages. For decades, ASD has been considered a disorder diagnosed based on behavioral principles or a symptom-based diagnosis (APA 2000). More recently, specific behavioral and interview procedures have created to lend objectivity to the diagnosis. These have included the Autism Diagnostic Observation Schedule (Lord et al. 2001) and Autism Diagnostic Interview—Revised (Le Couteur et al. 2003), which have demonstrated predictive diagnostic classifications in the 83–92 % ranges depending on the age of the child. These are largely considered the "gold standard" in the diagnosis of autism or autism spectrum cases.

Recently, there have been attempts to use EEG data to discriminate between autistics and healthy controls. Catarino et al. (2011) examined the role of EEG complexity in 15 autistics and 15 controls. There were significant differences between the groups with the autistic spectrum children showing less complexity. Duffy and Als (2012) recently studied a large cohort of children in which they compared EEG data between ASD and healthy controls. Looking at multivariate, principal components analysis coherence data, they were able to discriminate the groups. They demonstrated and confirmed short-distance hypercoherence and long-distance hypocoherences in the ASD sample. Overall, their classification success rate was between 86 and 88 %. Lastly, Ahmadlou et al. (2012) used a method called fuzzy synchronization to examine connectivity differences between autistics and controls. There was a 95 % discrimination rate but their sample size was quite small. Nevertheless, these studies have begun to show that EEG coherence data may be used as a neurophysiological basis to discriminate ASD from controls and this may help in the identification of these children. The rates at which these markers can predict group membership rival the "gold standards" in the field.

In our study reviewed above, we have shown that EEG variables can be used to discriminate between children on the autistic spectrum and normal controls. The sensitivity of this measure was 95.6 %, the specificity 92.3 % and the positive predictive value 95.5 %, and negative predictive value 92.6 %. These diagnostic accuracy rates compare quite favorably to the current "gold standards." Along with Duffy and Als (2012) study above, this is the first time that a physiological measure has been demonstrated to show such accuracy for autistic disorders. Because the measures used in this study to substantiate the ASD diagnosis fall

short of the highest degree of completeness, these results are best understood as preliminary and in need of replication.

Both the ability to discriminate autistic from normal children and the delineation of EEG subtypes of autistic disorder support the utility of the EEG in this condition. Such an analysis of differences in the type and pattern of power abnormalities has been useful in quantitative EEG research in ADHD (Chabot et al. 2005) and obsessive–compulsive disorder (Prichep et al. 1993). It has also been shown that the use of EEG power and connectivity data enhances the efficacy of EEG biofeedback for autistic children (Coben and Padolsky 2007; Coben and Myers, 2008a). The current study is, by far, the largest EEG study of autistic children. There were clear power and connectivity anomalies, discriminative power that rivals the "gold standards" in the field, and electrophysiological subtyping that may prove useful for future research and treatment planning. Future work is clearly needed to replicate these findings, test the power of the discriminant function to classify independent samples of normal and ASD children, and detail the source localization of these dysfunctions and enhance the regional resolution of connectivity findings.

The results of the VARETA analyses suggest that despite different patterns of EEG frequency abnormality across ASD subtypes, a single underlying neurophysiological pathway or network can be identified that shows dysfunction in ASD. When processed using the VARETA software, all five QEEG subtypes showed similar patterns of subcortical and cortical abnormality. VARETA images of ASD children revealed functional abnormality within the thalamus, hippocampus, and caudate nucleus that spread to and included the posterior cingulate, supramarginal gyrus, lateral and medial occipital/temporal, superior parietal, and occipital cortical regions bilaterally. The subcortical and cortical regions showing abnormal neurophysiological function in ASD children identified using QEEG-based VARETA imaging agree with the findings based upon other neuroimaging techniques such as MRI, fMRI, and PET.

Neuroimaging studies of ASD suggest abnormal function between frontal/striatal systems and more posterior cortical regions. This involves the disruption of the frontal/striatal and parietal networks important in the social brain system (McAlonan et al. 2005) and disruption of communication between the frontal/striatal, cerebellum, basal ganglia, thalamus, and ventral striatum important in mental-state attribution and the superior temporal region important in perception and eye gaze (Waiter et al. 2004), and decreased gray matter in frontal/temporal and somatosensory regions involved in social cognition (Rowe et al. 2007). Further studies in ASD note disruption of the connections between the posterior cingulate region and the inferior and ventral temporal regions involved with the integration of visual and affective information (Barnea-Goraly et al. 2004), decreased activity in the superior temporal region and the cerebellum involved in the integration of sensory and limbic information and social perceptual skills (Boddaert et al. 2004), and decreased caudate nucleus volume and repetitive behavior (Hollander et al. 2005).

QEEG and VARETA can play an important role in identifying the underlying neurophysiological abnormality present in ASD. Individual patterns of findings may have implications for diagnostic purposes as well as for treatment selection and

implementation. For example, individual QEEG frequency profiles and analysis of coherence patterns can be used to guide neurofeedback to reduce the salient QEEG abnormality, a treatment recently shown to have promise in ADHD and ASD (Monastra et al. 2005; Coben and Padolsky 2007). Neuropharmacotherapy can also use various pharmacological agents guided and assayed by their ability to normalize the QEEG which, from other pharmacokinetic studies, will predict favorable clinical responses (Saletu et al. 2006). Clearly, the findings reviewed in this chapter have implications for the diagnosis, assessment, and treatment of children on the autistic spectrum. We hope that future work in all of these areas progresses at a rapid pace.

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Biography

Robert Coben, Ph.D., received his doctoral degree in 1991 and has been a licensed psychologist in the state of New York since 1994. He is the Director and Chief Neuropsychologist of NeuroRehabilitation and Neuropsychological Services. His post-doctoral training in clinical and rehabilitation neuropsychology was done at the UCLA Medical Center and Cedars-Sinai Medical Center in California. His experience in rehabilitation neuropsychology includes directing two separate inpatient neurorehabilitation programs. He is former director of inpatient and outpatient brain rehabilitation at Staten Island University Hospital. He is an affiliate of Winthrop University Hospital and an affiliated researcher of NYU Medical Center.

Dr. Coben is a member in good standing of the American Psychological Association, International Neuropsychological Society, International Society for Neurofeedback and Research, and the American Association of Psychophysiology and Biofeedback. He is an associate editor for the Journal of Neurotherapy and Frontiers in Child Health and Human Development. He is also a editorial reviewer for the following journals: Journal of Neurotherapy, Journal of Autism and Developmental Disorders, Frontiers in Child Health and Human Development, Clinical Neurophysiology, Neuroimage, and Journal of Psychophysiology. He has edited special issues of journals on EEG Connectivity and more recently an upcoming issue on Applied Neuroscience, Neuromodulation and Neurofeedback. He has also edited a book entitled Neurofeedback and Neuromodulation Techniques and Applications. His research interests include the study of Neuropsychology and Neurophysiology in the understanding of childhood neurodevelopmental disorders, especially autism, and treatment applications for the same.

Robert J. Chabot, Ph.D., (research associate professor), has been a member of the research staff at the Brain Research Laboratories, New York University School of Medicine for the past 25 years. He has been involved in research designed to document the clinical utility of Quantitative EEG in neurological and psychiatric patient populations. Recently, he has been involved in the development of QEEG as a clinical tool for aiding in the diagnosis and treatment of children with attention deficit disorder, learning disorders, and those with autistic spectrum disorder. This research has been published in Biological Psychiatry, Journal of Child Neurology, and the Journal of Neuropsychiatry and Clinical Neurosciences with a comprehensive review paper in Adolescent Psychiatric Clinics of North America. Currently, he is involved with the development of a QEEG-based index that is sensitive to the detection of concussion and traumatic brain injury in emergency department patients.

Laurence Hirshberg, Ph.D., is a licensed clinical psychologist and serves on the faculty of the Department of Psychiatry and Human Behavior of the Brown University Medical School as clinical assistant professor. Dr. Hirshberg recently served as guest editor and contributor to a special issue of Child and Adolescent Psychiatric Clinics of North America devoted to emerging interventions in applied neuroscience, including neurofeedback and other brain-based interventions.

Dr. Hirshberg is conducting research on genetic, neurophysiological, and cognitive factors that predict drug treatment response and non response in individuals with major depression. The NeuroDevelopment Center is one of 20 neuroscience research sites in the USA, Canada, UK, South Africa, New Zealand, The Netherlands, and Australia selected to participate in this study. This study is the largest study of neurophysiological markers for depression that has ever been conducted. Dr. Hirshberg has been specializing in work with neurodevelopmental disorders for over 15 years and consults and trains educators and clinicians across New England. Dr. Hirshberg has published and presented in many areas of clinical psychology and child development.

Chapter 13
Behavior Imaging®'s Assessment Technology: A Mobile Infrastructure to Transform Autism Diagnosis and Treatment

Ron Oberleitner, Gregory Abowd, and Jasjit S. Suri

Much can be learned about autism through brain imaging. However, most decisions concerning the diagnosis of autism or the effectiveness of any given treatment are based on behavior data collected in natural environments. A growing body of research shows that collecting and analyzing behavior data in natural environments, such as schools and homes, provides data that supports current evidence-based decision practices for diagnosis and treatment. Behavior Imaging gives researchers and clinicians tools to collect rich, environmentally contextual data, enhancing diagnosis and treatment of individuals with autism spectrum disorder and related developmental disabilities. We will define Behavior Imaging and explain its development, its uses in treatment, and especially due to recent advances to include mobile devices, what the technology means for the future of autism diagnosis, treatment, and research.

13.1 Behavior Imaging® Defined

In response to the increasing demand by healthcare professionals internationally to include behavior information more systematically in the diagnosis and management of neurological and developmental disorders, academic and industry partners

®Behavior Imaging is a Registered Trademark of Caring Technologies, Inc., dba Behavior Imaging Solutions (2010–2012)

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developed a new imaging technology which is now capable of providing comprehensive documentation of relevant patient behaviors. Behavior Imaging now has the potential of transforming the behavior health industry in a manner similar to the impact observed when medical imaging was introduced (e.g., X-rays, MRIs, CT scans). Modeled on radiography and teleradiology in terms of its technical and regulatory specifications, Behavior Imaging refers to the capture, analysis, and secure review of a person's behavior information via video and other electronic means. Behavior information is often collected on video from a person's home, classroom, or clinical setting; then, when appropriate, this data is securely shared between the patient, their caregivers and/or providers, and researchers. All of this is done without regard for the stakeholders' geographic locations (Oberleitner et al. 2010).

Currently available Behavior Imaging systems feature novel video capture, clinical annotation tools, and a personal health record platform to capture, store, and securely share behavior data among caregivers and healthcare providers.

13.1.1 Behavior Capture™

Developed at the Georgia Institute of Technology's College of Computing, it consists of a video capture technology which can be used in a home or an institutional environment and features a unique video buffering capability that documents relevant events that occur before, during, and after a behavior. This "After-the-Fact™" video capturing feature provides insight into causes or triggers of certain behaviors.

Behavior Capture™ can record data from before and after activation of the system using a small wireless remote control device an important feature its invetors refer to as select archiving. This allows therapists and caregivers to capture behaviors "before the event." The camera/computer system continually captures videos but does not commit it to memory until the remote control is pressed (Fig. 13.1). For example, a provider can set the system to record events that occurred 10 min before the remote is activated and also for an additional 15 min (for a total recording time of 25 min).

13.1.2 Behavior Connect™

Behavior Connect is a secure, web-based health record platform for users to organize, analyze, and share videos and other documents such as medical history, prescribed medications, rating scales, and questionnaires among patients, health providers, teachers, therapists, and other professionals. This technology also allows

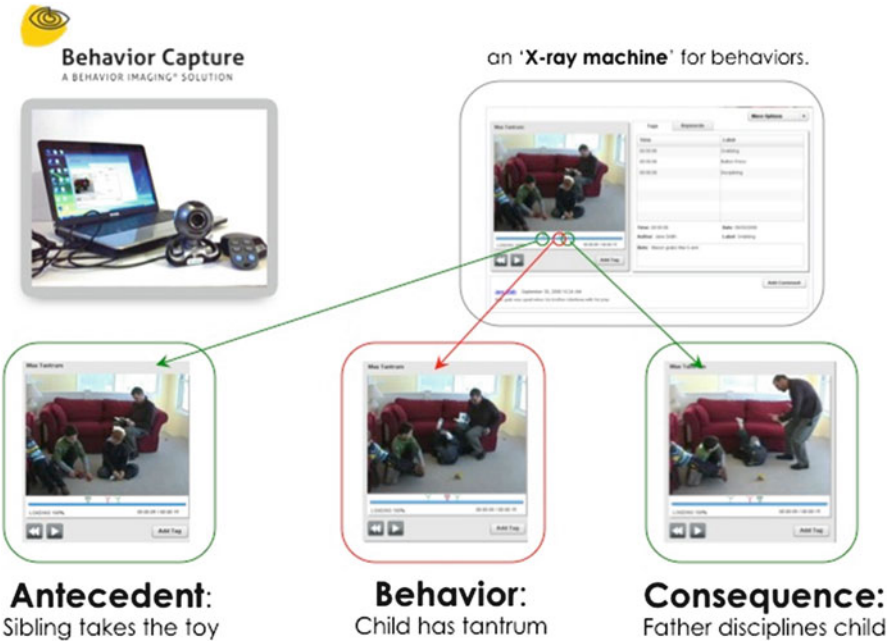

Fig. 13.1 Running on any PC, Behavior Capture™ uses After-the-Fact™ recording feature to enable a caregiver in a natural environment to capture a video clip of what happens before (antecedent), during (behavior), or after (consequence) with a small remote control device. That resultant video clip allows for the tagging of moments that either the caregiver or the health professional can later annotate for collaboration or data mining purposes

videos and other health data to be shared confidentially with family members or healthcare specialists via a secure, web-based consultation and records environment (Fig. 13.2).

13.2 Clinical Applications of Behavior Imaging

The utility of this approach has been evaluated in pilot studies for the capture of natural, spontaneous behaviors. It was found to improve the delivery of behavior therapy for autistic children (Oberleitner et al. 2004, 2005). The technology has also shown promise in the ability to more rapidly communicate changes in a child's behavior while reducing the wait for provider feedback (Oberleitner 2006). Researchers at Monash University (Melbourne, Australia) documented the benefits for autism families in rural areas of Australia. Behavior Imaging was a technology platform suitable for performing remote Functional Behavior Assessment (FBA) and provided a data sharing system that successfully linked rural families with behavior specialists in Melbourne (Thomas et al. 2009).

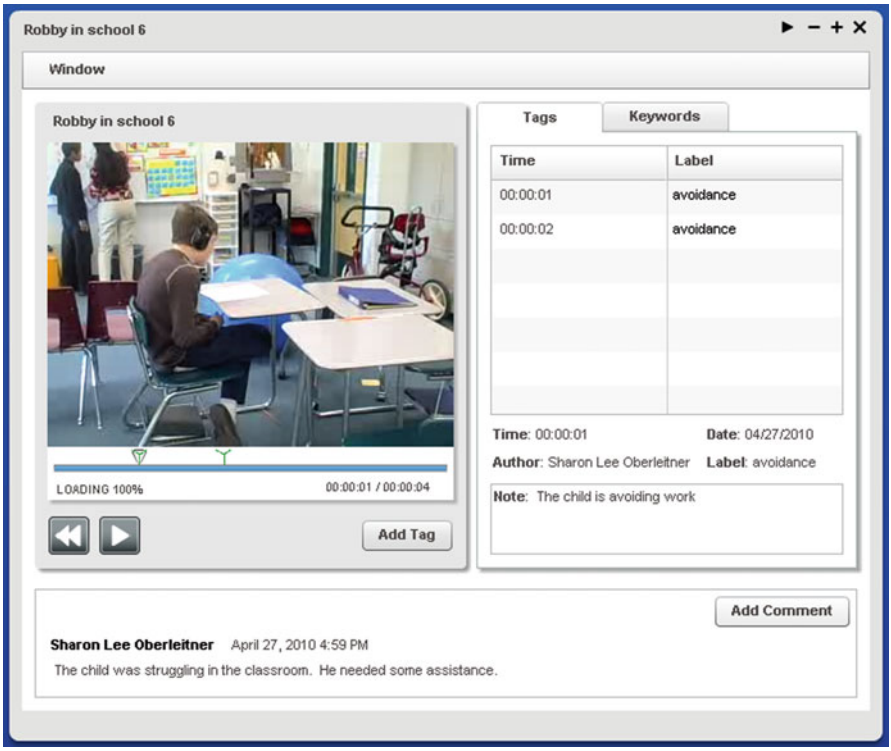

Fig. 13.2 This online data viewer of Behavior Connect™ allows clinicians to play and "tag" their clinical decisions directly on the video, and the clinician can time stamp any clinical comments like teleradiology systems do

13.2.1 Behavior Assessment

Children with ASD and other developmental disabilities exhibit behaviors that may be considered inappropriate and can even be dangerous to themselves or others. Unfortunately, many of these children cannot explain what leads them to perform this behavior (e.g., why they are hitting themselves and others), both because they may be nonverbal and because they may not have the capabilities to monitor and make sense of their internal states. Addressing these problem behaviors requires the collection of information about the underlying role and utility of these behaviors. This data collection occurs through a variety of approaches. One such approach, Functional Behavior Assessment (FBA), is commonly employed for understanding and addressing problem behavior (Horner and Carr 1997). Direct observation—in clinics, homes, and schools—is considered the gold standard for problem behavior assessment for

individuals with autism. However, it can be costly, intrusive, and ineffective because the presence of an unfamiliar observer causes children to change their behavior. In addition, families often endure long waiting lists to schedule appointments with specialists in the clinic or at school. Researchers at Georgia Institute of Technology developed selective archiving as an approach to video capture that provides an alternative to direct observation for naturalistic data capture. Selective archiving has been used to support functional behavior assessment in special education settings and in the collection of severe behavior data in homes (Hayes et al. 2008).

In one research study, four special education classrooms were equipped for 5 months as part of quasi-experimental study of Behavior Imaging's effectiveness. Four teachers conducted an FBA for each of their two students, one using the traditional pen-and-paper method and one using the new technology. The ordering of the two methods was counterbalanced and groups randomly assigned. To establish ground truth data on number of behavioral incidents, a member of the research staff recorded an average of 17 h or 21.5 % of time in study for each student with a handheld video camera. Independent coders then coded the videos for behavioral incidents using operational definitions created by the research team and teachers. With Behavior Imaging, teachers made an average of 43.37 % fewer false-negative errors (behavior incidents missed) than with the traditional pen-and-paper method. There were almost no false positives in either case. Also, given the ability to go back to replay the video as often as they like, the observations and interviews with teachers indicated that they felt more confident in their notation of antecedent and consequence data in the Behavior Imaging condition (Abowd et al. 2012).

13.2.2 Diagnostic Evaluation

Early work by the University of Medicine and Dentistry of New Jersey (UMDNJ) and Princeton Autism Technology (PAT) revealed that Behavior Imaging provided valid diagnostic data directly from the home of the autism families. Families learned about how to administer diagnostic tests by watching training video and then successfully video captured themselves, administering the test to their child. The diagnostic doctors were able to detect helpful diagnostic data from several video captures. A subsequent study conducted by the Southwest Autism Regional Resource Center (SARRC), Arizona, an autism centerserving 11,000 individuals annually, showed that the use of Behavior Imaging accelerated the diagnosis of autism in a carefully monitored pilot study. These and other projects demonstrate the potential that Behavior Imaging can facilitate autism diagnosis for selected subtypes of autism, provide more accurate and more contextual information relevant to the management of the health condition, and serve as a resource by providing the ability to archive all relevant data in a child's electronic health record.

13.2.3 Other Clinical Applications

Many other applications have also been successfully demonstrated. These can range from being used in new ways such as web-based supervision of staff who work with children with autism to assessment and therapeutic documentation.

13.3 Future of Diagnosis: SmartCapture™ and Diagnostic Lab Service

The advent of powerful, inexpensive smartphones has paved the way for the next generation of Behavior Imaging devices. This moves the power of Behavior Imaging to work on a truly mobile infrastructure—and away from more stationary PC-based systems. Known as SmartCapture™, this mobile device generation will offer an array of new specialty services in Behavior Connect and Behavior Capture. After validation in clinical trials, these services will demonstrate that remote child development specialists need not visit certain patients to make clinical observations based on imaging data they receive remotely. As illustrated below in Fig. 13.3, a new research study funded by the National institutes of Mental Health (NIMH) will assess effectiveness of the clinical workflow enabled by Behavior Imaging on mobile devices:

1. From a referral from the family's pediatrician, a concerned family can download a SmartCapture application on their smartphone. This will provide them a detailed "prescription" of how to capture video recordings of their child's activities and details regarding what episodes they are to capture.
2. The videos captured by caregivers will be automatically uploaded to the Behavior Connect online consultation platform, where they can be reviewed by a diagnostic clinician from a remote child development laboratory. These clinicians can solicit additional recordings as needed through a secure message system.
3. The clinician will review and tag the videos using DSM-IV criteria (a reference checklist used by physicians) if they observe evidence of developmental delay. The platform will automatically categorize these clinical observations and produce a report showing a DSM-IV checklist. In addition to this checklist, it will display actual evidence-based video examples of the observed developmental delay.

These reports can be forwarded to any authorized health practitioner as a test result. That practitioner will then solicit an expert second opinion to accelerate an autism diagnosis, if one is warranted. They could then refer the patient to a specialist or help the patient's family understand that the test results are demonstrating a negative result for developmental delay. The availability of this imaging service could transform the currently laborious process for diagnosing autism for many individuals who may be clear candidates—and are especially in underserved areas without the availability of diagnostic specialists available to assist local caregivers.

Fig. 13.3 For effectively capturing, analyzing, and reporting Behavior Imaging data, the following clinical workflow is followed (1) Specialists prescribe and receive BIs from caregivers—who use SmartCapture devices. (2) They review BIs in child development laboratories. (3) These specialists solicit more data, may refer to other specialties, and/or document their findings via special diagnostic reports

13.4 Conclusion

The use of electronic imaging to improve diagnosis and treatment is in its early stage. There are many exciting opportunities to use this imaging technology to increase access to healthcare and diagnostic services for individuals with autism and better understand the conditions of autism than current methods allow.

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Biography

Ron Oberleitner has been principal investigator on several NIH research studies to evaluate the use of imaging and telehealth technology to improve autism diagnosis, treatment, and assessment. His expertise includes leading technology research and development in fields of telehealth, medical device, and health record technology. He has successfully commercialized numerous technological innovations for the autism community, including Behavior Imaging®, TalkAutism, and AutismCares. He is a leading expert and published researcher on the use of health informatics, assessment tools, and telemedicine for special needs population, and is expanding positive findings to research new applications in dementia, depression, and post-traumatic stress disorder. He is also the father of a 20-year-old son with autism.

Gregory Abowd, Ph.D., is a Regents' and Distinguished Professor in the School of Interactive Computing at the Georgia Institute of Technology. Dr. Abowd leads a research group interested in human-centered applications of mobile and ubiquitous computing technologies, with an emphasis on home and health. He established the Aware Home Research Initiative at Georgia Tech in the late 1990's and has also researched applications of technology in the classroom. He was a leader in establishing the importance of computing technology to address a variety of challenges linked to autism, and has published widely on this topic and assisted in the

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