Nadja Reissland · Barbara S. Kisilevsky
Editors

Fetal Development

Research on Brain and Behavior, Environmental Influences, and Emerging Technologies

Foreword by
Gerard H.A. Visser and Eduard J.H. Mulder

Springer

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Foreword

Development begins before birth with the foundation for short and long term outcomes being laid during gestation, making the prenatal phase probably the most important period of our structural and functional growth and maturity. It takes 42 cell divisions from conception to birth and it is obvious that many factors may influence prenatal development during these numerous divisions. Clearly, studies during the prenatal period are essential for the understanding of normal and altered development, of the consequences for later life and for generating strategies to ensure optimal prenatal development.

Over the years, since the introduction of ultrasound, there have been numerous scientific articles, proceedings, and edited books on fetal behavioral development which today provide a historical perspective on the emergence and transition of the field. The following is a sample selection of edited books containing a range of foci of fetal research for illustrative purposes. One of the early books entitled *Continuity of neural functions from prenatal to postnatal life* (1984) was edited by Heinz Prechtl. Subsequently, Nijhuis (1992) edited a volume entitled *Fetal Behaviour: Developmental and perinatal aspects* which was followed shortly thereafter by Lecanuet, Fifer, Krasnegor, and Smotherman's (1995), *Fetal Development: A psychobiological perspective*. The most recent publication (2012), again, by Prechtl and others was entitled *Fetal behaviour: a neurodevelopmental approach*. These earlier collections as well as the current one demonstrate the impact that numerous research laboratories worldwide have had on the understanding of fetal behavior and neurological development, advancing the field in a relatively brief period of time.

Bringing research on fetal brain and neurological development together is important for several reasons:

1. First, it shows the miracle of early development of fetal behavior in normal pregnancies. At around 14 weeks of gestation almost all movement patterns observed in newborn infants are already present and executed more or less similarly, taking into account prenatal spatial restrictions and the absence of gravity. These movements are necessary for muscular development, for shaping central nervous system (CNS) structures, for in utero survival (e.g., fetal swallowing to prevent polyhydramnios) and for adaptation to neonatal functioning (e.g., breathing movements). In the course of pregnancy, rest-activity cycles develop and at the end of pregnancy fully developed fetal behavioral states are present, similar to the

ones of the newborn, and with a cycle length of REM and non-REM sleep comparable to that of adults. Hence, there is continuity from prenatal to postnatal life. Alterations in fetal blood flow distribution associated with sleep states show the high level of organization before birth.
2. Knowledge of fetal behavior is important for the assessment of fetal health. This holds for the interpretation of fetal movement patterns, assessment of fetal heart rate patterns, with prolonged episodes of low fetal heart rate variation as part of non-REM sleep and for the understanding of the absence of fetal reactions to external stimulation during non-REM sleep.
3. Knowledge of the quantity and quality of fetal movements and of the development of fetal behavioral states facilitates assessment of CNS integrity. Moreover, it allows investigations of altered fetal development in cases of pregnancy complications such as maternal diabetes. Similarly, the effect of fetal growth restriction may be studied and the effects of maternal stress and emotions, external stimuli, medication, drugs, and alcohol. This is the field of fetal behavioral teratogenicity.
4. Finally, fetal behavioral studies facilitate research linking disturbances in prenatal development to long-term outcome, as part of the Developmental Origins of Health and Disease (DOHaD).

In this book the following issues are addressed: (novel) techniques to study fetal behavior including functional MRI and fetal heart rate patterns; the normal development of fetal behavior; assessment tools to study CNS integrity; numerous examples of altered intrauterine development due to behavioral teratogens such as maternal alcohol ingestion; studies linking abnormal development to impaired later outcome; and finally chapters addressing the importance of animal models for studying prenatal behavioral development. In observational studies in the human it remains difficult to attribute abnormal development to either external influences for instance due to stress or medication or genetic background, whereas in animal studies a randomized controlled design allows for unequivocal study of the former.

We would like to congratulate Barbara Kisilevsky and Nadja Reissland for the great effort they undertook to convince many distinguished researchers to contribute a chapter to this book. It has resulted in an overview of the whole spectrum of fetal neurological, brain, and behavioral development: a must for everyone interested in the beginning of development and on factors that may either facilitate or hamper that development.

Utrecht, The Netherlands

Gerard H.A. Visser
Eduard J.H. Mulder

Preface

This edited volume was motivated by the need for an updated overview of the psychobiological study of fetal development, bringing together information from diverse areas into a single source. The field has a relatively brief scientific history, with systematic study only beginning in the latter part of the twentieth century. While observations of behavior (heart rate and body movements) and behavior change had been used in earlier work, the introduction and ready availability of ultrasound equipment with sophisticated image processing techniques provided a window into the womb, partially addressing the challenges of inaccessibility. Research theories and methods advanced along with technology as confirmatory evidence of the influential effects of the prenatal period on long-term outcome emerged. To illustrate the state-of-the-science, chapters provide historical appraisals, summaries of questions that have been addressed, and of discoveries that have been made in both human investigations and animal models. The historical overview is complemented with potential directions for future research considering the paths that could, or should, or might yet be taken.

The chapters are divided into three broad sections: brain-behavior development, environmental influences, and emerging technology. Chapters focusing on fundamental brain-behavior development and functioning are further divided into two parts. The first part includes those using animal models ranging from mouse to chimpanzee. Insights gained from studies of various animal models employ methods which cannot be used with human participants to explain behavior. The importance of the use of animals is reflected in data collected under strictly controlled conditions which are essential in generating theories of early perceptual, cognitive, and social development explained with biological and neurophysiological mechanisms. The second part includes studies examining human fetuses. Observations have identified the onset and characterized the maturation of spontaneous behaviors including heart rate, its variability and patterns, as well as body, limb, eye, breathing, and mouth movements. Over gestation, there is increasing coordination among these behaviors resulting in organized behavioral states. Development of the sensory systems, in particular audition and touch, is illustrated, employing changes in behavior, elicited by acoustic and/or vibroacoustic stimuli. Behavior is described not only for fetuses developing in a normal pregnancy but also differential development in groups subjected to atypical environments. These studies are designed to ultimately play a role in identifying clinical markers of neuropathology. Preliminary work on the use of behaviors

in the assessment of fetal well-being is explored in various chapters throughout the book.

The second section includes chapters which investigate environmental influences on short- and long-term developmental outcome. This section also is divided into two parts, with researchers in the first examining the fetal effects of maternal emotions during pregnancy and in the second, identifying the consequences of iron deficiency, maternal alcohol ingestion, and serotonin reuptake inhibitor exposure. The influence of maternal mental health including maternal stress, depression, and anxiety is attributed by some to fetal "programming." Studies of iron deficiency and alcohol teratogenicity assess links between genetic and epigenetic factors leading to negative neurodevelopmental outcomes. Studies of serotonin reuptake inhibitors question whether the effects reflect an acute short-lived pharmacological phenomenon or sustained neurological changes associated with neurotransmitter signaling.

The final section describes new technologies, the issues, and promise they hold for directly linking human brain activity and behavior. Fetal magnetic resonance imaging and magnetoencephalography permit direct determinations of brain structure and function with the latter measuring fetal neuronal activity and precisely determining brain development. Magnetocardiography allows for measuring the effects of interventions on the development of fetal neurobehaviors and cardiac autonomic control. Incorporating HD live software into ultrasound holds the promise of facilitating future behavioral research on twins.

The thematic sections provide a means of organizing the content of this volume. Given that the chapters cover a gamut of developmental issues from gene expression, neurotransmitter synthesis and metabolism, brain structure/growth, neurobehavior, and neurocognition as well as nutritional, pharmacological, psychological, and social factors associated with specific behaviors within the context of developmental vulnerability and neuroplasticity, the diversity of research questions and methods cross traditional boundaries. Thus, research in any one chapter is not mutually exclusive to the particular theme. Overall, the book provides the reader with a historical perspective of fetal psychobiological research, a current overview of findings, and proposals for future research directions (see Afterword). The diversity of issues included in this volume demonstrates the importance of research on prenatal development from a multidisciplinary approach including animal models and human fetuses, incorporating genetic, epigenetic, behavioral, and sophisticated imaging research techniques.

Kingston, ON, Canada — Barbara S. Kisilevsky
Durham, UK — Nadja Reissland

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About the Editors

Nadja Reissland, BSc, MA, DPhil (Oxon) is a Developmental Psychologist, Associate Professor in the Department of Psychology and Deputy Head of the Science Faculty, Durham University, UK. Her work is at the forefront of the growing field of fetal psychology and is especially focused on fetal development in relation to maternal stress and depression, as well as early mother-infant interaction. Her pioneering research on fetal movements, specifically fetal facial movements, has potential applications for obstetricians helping to identify indicators of healthy development in utero. Her research program includes the effects of maternal mental health (stress and depression) as well as health behaviors (smoking) on fetal behavior. Furthermore she evaluates behaviors including laterality, vision, and precursors to language as evidence of the development of CNS function.

Barbara S. Kisilevsky, BN, MN, MA, PhD is Professor Emerita, Queen's University, Kingston, ON, Canada. With academic appointments in Nursing, Psychology, and Obstetrics & Gynaecology, she established an internationally recognized multidisciplinary research program in the area of sensory development in the perinatal period with collaborations in the USA, Europe, and Asia. In over 30 years of examining behavior (e.g., cardiac, body movements) from a psychobiological perspective, she together with trainees and collaborators has characterized auditory (e.g., white noise, music, mother's voice) and vibroacoustic sensitivity in fetuses in uneventful pregnancies over mid- to late gestation; identified differential behaviors in fetuses in pregnancies complicated by conditions associated with placental insufficiency; determined sensory interventions to reduce behavioral and physiological responses of premature infants to "pain" stimuli; described mother-infant interactions cross-culturally; and, demonstrated a relationship between fetal voice/speech processing, newborn information processing, and infant language ability. Her laboratory has served as a training facility for international visiting scientists, PhD, MSc, and MA thesis students, and numerous undergraduate summer students.

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Part I

Brain-Behavior Development: Animal Models

Using an Animal Model to Explore the Prenatal Origins of Social Development

1

Robert Lickliter and Lorraine E. Bahrick

Abstract

Prenatal experience is both a formative and regulatory force in the process of development. As a result, birth is not an adequate starting point for explanations of behavioral development. However, little is currently known regarding the role of prenatal experience in the emergence and development of neonatal social orienting, social motivation, or social learning. Our lack of knowledge in this area is due in part to the very restricted experimental manipulations possible with human fetuses. A comparative approach utilizing animal models provides an essential step in addressing this gap in our knowledge of the development of social responsiveness and providing testable predictions for studies with human fetuses and infants. In this chapter we review animal-based research exploring how aspects of prenatal experience can facilitate the development of postnatal social motivation, social recognition, and social learning. We conclude that infant social responsiveness has its roots in prenatal development and that intersensory redundancy present in the prenatal environment promotes the salience of social stimuli during early postnatal development.

Keywords

Prenatal learning • Intersensory redundancy • Origins of social development • Behavioral embryology • Selective attention

Evidence obtained over the last 40 years with human infants indicates that social stimuli such as faces and voices are typically preferred over other stimuli even in the days immediately following birth (e.g., Goren, Sarty, & Wu, 1975; Legerstee, Pomerleau, Malcuit, & Feider, 1987; Maurer & Young, 1983; Valenza, Simion, Cassia, & Umilta, 1996). As a result of the early salience of faces and voices to infants, some developmental psychologists have proposed that neonates' biases or preferences towards social stimuli are

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innate, in that they are present at birth and do not require prior experience (e.g., Balas, 2010; Gergely & Watson, 1999; Meltzoff & Decety, 2003; Muir & Nadel, 1998). However, a growing body of comparative evidence indicates that prenatal experience plays a key role in establishing early postnatal perceptual preferences (Gottlieb, 1997; Harshaw & Lickliter, 2011; Hopkins & Johnson, 2005; Lickliter, 2005; Schaal, Marlier, & Soussignan, 1998). Whether and to what extent prenatal sensory experience influences early social development however remains relatively unexplored (but see DeCasper & Fifer, 1980; Mastropieri & Turkewitz, 1999; Moon, Panneton-Cooper, & Fifer, 1993 for suggestive examples from human neonates).

The use of animal models offers an essential step in addressing this critical gap in our knowledge base. Studies that manipulate human fetuses and infants are necessarily severely constrained and animal based research serves to minimize the amount of exploratory research undertaken with human participants and hone in on issues and directions worthy of further research investment (Gottlieb & Lickliter, 2004; Lickliter & Bahrick, 2000, 2007). In particular, the use of animal models allows the collection of data under more strictly controlled conditions than would be possible with human fetuses and infants and is thus key for eventually connecting theories of early perceptual, cognitive, and social development with biological and neurophysiological mechanisms.

Of course, we cannot answer questions about human development by primarily studying animals, but comparative work can provide new questions, methods, and potentially derive developmental principles that can then be tested with humans. As pointed out by Arnold and Spear (1997), the determinants of early perception and cognition are too basic to consider them solely with tests of humans. The utilization of interdisciplinary, comparative, and convergent research strategies is a critical step in discovering and defining the various conditions, experiences, and events (both internal and external) necessary and sufficient for normal perceptual, cognitive, and social development. This approach can also shed light on the conditions, experiences, and events that contribute to atypical development.

The Avian Model for Studying the Role of Experience in Prenatal Development

The embryonic bird develops entirely within the egg, externalized from the mother, thereby providing a well-controlled "laboratory" for introducing experimental manipulations into the prenatal environment. Precocial birds (e.g., chickens, ducks, quail) allow easy access to the embryo for prenatal observation and manipulation. Further, their developmental age and experiential history can be precisely controlled since they can be incubated in the laboratory, and they have several important similarities to human sensory organization. Like humans, in precocial birds all five sensory modalities are functional in the late stages of prenatal development. Further, like humans, avian embryos can learn the acoustic features of maternal vocalizations prior to hatching (Bailey & Ralph, 1975; Gottlieb, 1971; Heaton, Goodwin, & Miller, 1978). Unlike humans, however, precocial birds have the advantage that they can demonstrate perceptual and social preferences by means of their locomotor behavior in the days immediately following hatching. Leveraging these unique developmental conditions, research has consistently demonstrated that specific features of prenatal sensory experience, particularly the temporal synchrony of multisensory stimulation, can significantly influence embryos' and chicks' arousal, selective attention, perceptual learning, and memory (e.g., Jaime, Bahrick, & Lickliter, 2010; Lickliter, Bahrick, & Honeycutt, 2002, 2004; Lickliter, Bahrick, & Markham, 2006; Reynolds & Lickliter, 2002, 2003). In this chapter, we review this body of research and discuss its implications for the development of social responsiveness during early development. We also explore the usefulness of animal-based research for better integrating the prenatal period into theories of both typical and atypical development.

Exploring the Ecology of Prenatal Sensory Experience

One obvious advantage of the use of animal subjects to study perceptual, cognitive, and social development in the prenatal period is the ability to readily alter both the timing and amount of particular sensory experience available to the developing embryo or fetus. In contrast to mammals where the fetal environment is difficult to access and manipulate, the avian egg environment can be experimentally manipulated independently of the mother and thus provides an accessible means for testing hypotheses about prenatal factors underlying subsequent postnatal development. For example, the avian embryo can be exposed to premature visual stimulation or augmented auditory stimulation by the simple procedure of removing the upper portion of the egg-shell several days prior to hatching, thereby exposing the head of the embryo to external stimulation. As a result, it is possible to readily employ sensory augmentation, sensory deprivation, or sensory substitution techniques during the prenatal period. This approach of modifying typical patterns of prenatal experience has provided a large body of evidence regarding the experiential conditions necessary for the normal development of early sensory organization and perceptual development (e.g., Gottlieb, 1971, 1997; Honeycutt & Lickliter, 2003; Markham, Shimizu, & Lickliter, 2008; Radell & Gottlieb, 1992; Sleigh & Lickliter, 1998). Taken together, this research indicates that the specific effects that prenatal sensory experience can have on early perceptual development and sensory integration depend on a number of interrelated factors, including (a) the *timing* of sensory experience, (b) the *amount* of sensory experience, and (c) the *type* of sensory experience encountered by the avian embryo or mammalian fetus (reviewed in Lickliter, 2000, 2005).

Timing of Prenatal Sensory Stimulation

All the sensory systems begin to develop prenatally in birds and mammals and, in precocial species (including humans), they are capable of functioning before birth. The prenatal environment is thus rich in tactile, vestibular, chemical, and auditory sensory stimulation. However, as first pointed out by Gottlieb (1971), at birth the sensory systems are not on equal footing. This is the case because the onset of function within the various sensory modalities does not occur at the same time in prenatal development. Rather, the sensory systems become functional in a specific and invariant sequence across early development: tactile > vestibular > chemical > auditory > visual (Alberts, 1984; Bradley & Mistretta, 1975; Gottlieb, 1971). Further, the tactile and chemical senses are comprised of many kinds of sense receptors, which have their own timing of development. As a result, because of the timing of their onset of function, the various sensory modalities of birds and mammals have markedly different developmental histories at the time of hatching or birth. For example, at birth the earlier developing tactile and vestibular systems have had much more experience than the later developing auditory system. These temporal dynamics likely have significant consequences for the course of early postnatal perceptual development and much remains to be learned about links between the order and timing of prenatal sensory experience and subsequent postnatal perceptual processing.

Turkewitz and Kenny (1985) proposed that the differential timing of sensory system onset provides a restrictive context in which earlier developing sensory systems can develop without competition or interference from later developing sensory systems. If this is the case, it would have important implications for the care and management of preterm human infants (Lickliter, 2000, 2011). The limited sensory capacities of the fetus (as a result of the staggered onset of sensory function across prenatal development) and the constrained and buffered developmental context of the uterus combine to effectively limit and regulate the amount, type, and timing of sensory stimulation available to the fetus during the prenatal period. However, these limited and regulated patterns of sensory stimulation are dramatically disrupted by preterm birth. Infants born weeks or even months before term are routinely exposed to altered amounts, types, and timing of sensory stimulation as compared to

full-term infants. For example, the preterm infant housed in the neonatal intensive care unit (NICU) receives substantially decreased amounts of tactile and vestibular stimulation from maternal movement and substantially increased amounts of unfiltered auditory and patterned visual stimulation as compared to full-terms. The perceptual, cognitive, and social consequences of these modifications in movement, sound, and light are not well understood, but studies have suggested that the atypical sensory environment provided in the NICU can have enduring effects on the developing premature brain (e.g., Als et al., 2003; Aylward, 2005; Gressens, Rogido, Paindaveine, & Sola, 2002) and later behavior (Rand & Lahav, 2014).

One approach to examining the importance of asynchronous sensory development is to alter the time when particular sensory input would normally be present during the prenatal period. Using this approach, Lickliter (1990) found that the introduction of unusually early prenatal visual experience interfered with species-typical auditory responsiveness in bobwhite quail chicks following hatching. Chicks that experienced patterned light prior to hatching failed to exhibit the typical naïve preference for their species-specific maternal call, a reliable phenomenon observed in control chicks not receiving prenatal visual stimulation. An important implication of this finding is that prenatal experiential input to one sensory modality cannot be examined in isolation, as the effects of sensory experience not only influence the particular modality in question, but also other developing modalities as well. This finding of intersensory linkages is an important change of emphasis, as the different sensory systems have historically been studied individually. Vision, audition, touch, taste, and smell have been studied as if they operate without significant links to each other. We now know this is not the case, even during prenatal development (Bremner, Lewkowicz, & Spence, 2012; Calvert, Spence, & Stein, 2004).

Findings also indicate that modified prenatal stimulation to earlier-emerging sensory modalities can either facilitate or interfere with species-typical perceptual responsiveness in later-developing sensory modalities, depending on *when* the modified prenatal stimulation takes place. For example, differences in the timing of augmented prenatal stimulation to quail embryos led to different patterns of auditory and visual responsiveness following hatching. No effect on normal responsiveness to maternal visual cues was found when exposure to tactile and vestibular stimulation *coincided* with the emergence of visual function, but when exposure took place *after* the onset of visual functioning chicks displayed enhanced responsiveness to the same maternal visual cues when compared to controls not receiving modified sensory stimulation. When augmented tactile and vestibular stimulation *coincided* with the onset of auditory function, embryos subsequently failed to learn a species-typical maternal call prior to hatching. However, when given exposure to the same type and amount of augmented stimulation *following* the onset of auditory function, embryos did successfully learn the individual maternal call (Honeycutt & Lickliter, 2003). These findings provide evidence of the dynamic nature of sensory experience, as differences in the time of exposure results in differences in subsequent perceptual and cognitive development.

Research also indicates that modifications in the timing of patterns of prenatal sensory experience can have effects on early brain growth and development. For example, Markham et al. (2008) presented augmented amounts of auditory stimulation to bobwhite quail embryos during the early, middle, or late stages of prenatal development and then tested postnatal responsiveness to both maternal auditory and visual stimulation. Embryos receiving auditory stimulation during the *middle* or *late* stages of prenatal development showed atypical postnatal visual responsiveness to a bobwhite hen when compared to controls. These birds also showed a greater number of cells per unit volume of brain tissue in deep optic tectum, a midbrain region implicated in multisensory function. In contrast, embryos receiving modified auditory stimulation in the *early* stages of prenatal development did not show altered behavioral or neural development. These results indicate that modified sensory experience, such as those pro-

vided to human preterm infants by the NICU environment, can influence both perception as well as the trajectory of brain growth. These effects were temporally constrained; *when* the sensory modification occurred mattered. This principle of prenatal temporal constraints has likewise been found to be at play in the area of teratology, particularly in the well-known example of the time sensitive effects of fetal alcohol exposure.

Multimodal Stimulation During Prenatal Development

Sensory stimulation present in the prenatal environment is typically multisensory in nature. The prenatal environment provides the fetus a variety of concurrent tactile, vestibular, chemical, or auditory sensory information (DeCasper & Fifer, 1980; Hepper, Scott, & Shahidulla, 1993; Kisilevsky & Low, 1998; Smotherman & Robinson, 1986). Although little research has directly focused on this issue, the human fetus likely experiences a great deal of integrated multimodal stimulation across the auditory, vestibular, and tactile senses in utero. For example, when the mother walks, the sounds of her footsteps can be coordinated with tactile feedback as the fetus experiences changing pressure corresponding with the temporal patterning and shifting intensity of her movements, as well as accompanying and coordinated vestibular changes. In addition, the mother's speech sounds, laughter, heart beat, or sounds of breathing may create tactile stimulation that shares the temporal patterning of the sounds as a result of changes in the musculature involved in producing the sounds.

Fetuses also engage in spontaneous motor activity of limbs and body, providing temporally organized, cyclic self stimulation (Robertson & Bacher, 1995). When the fetus moves in the uterus, the movement generates temporally coordinated proprioceptive feedback as well as temporally coordinated tactile consequences of the motion, such as changes in pressure on the skin. The example of fetal thumb sucking well illustrates this coordinated pattern of multisensory self-generated stimulation. Additionally, the mother also responds with temporally coordinated movements to externally generated sounds. She may dance or exercise to music, startle to a loud noise, engage in conversation which has a distinctive turn-taking contingent structure, all of which produce movements that have tactile and/or vestibular correlates that share intensity and temporal patterning with the sounds. Thus, the fetus likely has ample opportunity from self stimulation and environmental stimulation to become familiar with and detect redundant stimulation across the various senses during the late stages of prenatal development. The role of this prenatal intersensory experience in typical perceptual, cognitive, or social development is likely significant but currently not well understood.

We do know that *infant* detection of *amodal* stimulus properties (information that is common across the senses) such as synchrony, intensity, rhythm, and tempo is promoted by multimodal redundancy across sensory systems and is involved in the emergence of normal patterns of perceptual organization (Bahrick & Pickens, 1994; Bremner et al., 2012). Importantly, the temporal and spatial aspects of stimulation are typically conveyed across multiple senses. For example, the rhythm or rate of a ball bouncing can be conveyed visually or acoustically and is completely redundant across the two senses. The sight and sound of hands clapping likewise share temporal synchrony, a common tempo of action, and a common rhythm. Even very young infants are adept perceivers of such amodal stimulation (e.g., Bahrick & Pickens, 1994; Lewkowicz, 2000; Lickliter & Bahrick, 2000). Infants as young as 2 months can detect the temporal aspects of stimulation such as synchrony, rhythm, tempo, and prosody that unite visual and acoustic stimulation from single events, as well as spatial co-location of objects and their sound sources, and changes in intensity across the senses (see Bremner et al., 2012; Lewkowicz & Lickliter, 1994 for reviews). Detection of amodal information in early development does away with the notion of young perceivers having to coordinate and put together separate and distinct sources of information. Our work with quail embryos has established that even when the amount of overall *prenatal* sensory stimulation is controlled, detection and learning of temporal

stimulus properties such as tempo and rhythm by embryos are facilitated in redundant bimodal stimulation (which highlights amodal information) as compared to unimodal stimulation (Lickliter et al., 2002, 2004).

Intersensory Redundancy and Social Responsiveness

In particular, we have found that *intersensory redundancy*, the same information simultaneously available and temporally synchronized across two or more sensory systems, facilitates embryos' prenatal learning of an individual maternal call. Lickliter et al. (2002) exposed quail embryos (that can perceive light and sound through the egg shell) to an individual maternal call for 6, 12, or 24 h (10 min/h, for a total of 60, 120, or 240 min of exposure) during the late stages of incubation. The maternal call was presented under conditions of (a) unimodal auditory stimulation, (b) concurrent but asynchronous auditory and visual stimulation (patterned light), or (c) redundant and synchronous auditory and visual stimulation, achieved by presenting the call with a light that flashed in synchrony and had the same temporal pattern (rate, rhythm, duration) as the notes of a maternal call. Following hatching, chicks from all conditions received a simultaneous two-choice preference test for the familiarized vs. a novel maternal call. We found that embryos exposed to the redundantly presented maternal call (auditory and visual) showed dramatic facilitation, learning the call four times faster and remembering it four times longer into postnatal development (4 days) than those exposed to unimodal auditory stimulation (1 day). Further, embryos that received nonredundant asynchronous call and light exposure to control for the overall amount of stimulation showed no evidence of learning the familiarized call following hatching, demonstrating no preference for either maternal call during testing.

We have also found that providing embryos intersensory redundancy during late prenatal development educates their attention to specific stimulus properties (Lickliter et al., 2006). Quail chicks showed no preference for a familiar maternal call after a brief prenatal unimodal auditory familiarization. In contrast, by first exposing embryos to a redundant audiovisual presentation (call synchronized with flashing light) followed by the unimodal auditory presentation (i.e., bimodal → unimodal), chicks preferred the familiar auditory maternal call 2 days after hatching. Embryos who received the reverse sequence prenatally (unimodal → bimodal) showed no preference for the familiarized call. This education of attention was effective even after delays of 2 or 4 h between initial bimodal exposure and subsequent unimodal exposure, and continued to affect learning and memory days later (Lickliter et al., 2006).

Studies of human infants have found parallel findings. For example, 4 month-old infants detect a change in the tempo of a toy hammer tapping in unimodal visual stimulation, but only if they receive a brief pre-exposure to the tempo in bimodally redundant (synchronous audiovisual) stimulation, thereby educating their attention to the tempo information. Infants fail to detect the tempo change following nonredundant (unimodal visual or asynchronous audiovisual) pre-exposure (Castellanos, Vaillant-Molina, Lickliter, & Bahrick, 2006). By educating their selective attention to amodal properties, both animal and human infants can continue to detect these amodal properties in the same events, even when redundancy is no longer available. This finding suggests that the education of attention can foster flexible processing and may serve as a mechanism for promoting developmental change in attentional selectivity, from detection of amodal properties in multimodal stimulation to detection of the same amodal properties in all types of stimulation.

Promoting Neonatal Social Responsiveness

Given the demonstrations of quail embryos' sensitivity to intersensory redundancy, as well as the documented sensitivity of human infants to intersensory redundancy (see Bahrick & Lickliter, 2002, 2012 for reviews), we are currently explor-

ing the relevance of this sensitivity to early social development. Social events are one of the first and most frequently encountered sources of intersensory redundancy both before and following birth or hatching, and we were interested in whether and to what extent the amount and type of intersensory redundancy available during prenatal development fosters social orienting, social learning, and social memory during early postnatal development. Social events provide high amounts of sensory redundancy relative to most nonsocial events. For example, parents provide social stimulation to their infant that contains a great deal of redundancy across tactile, auditory, and visual sensory systems. Audiovisual speech is rich with intersensory redundancy uniting the tempo, rhythm, and intensity shifts across faces and voices. We have hypothesized that this redundant multimodal stimulation can educate attention and foster the emergence of social orienting in early development by attracting and maintaining selective attention to faces, voices, and audiovisual speech. This could in turn promote early social development, as well as related perceptual and cognitive development (see Bahrick, 2010 for examples in typical and atypical developing infants).

Intersensory Redundancy Responsiveness

Building on our previous animal and human infant research on the role of intersensory redundancy in early perceptual and cognitive development (Bahrick & Lickliter, 2002, 2012; Lickliter & Bahrick, 2004), our working hypothesis is that exposure to multimodal *intersensory redundancy* (the same information simultaneously available and temporally synchronized across two or more senses) provided by the mother prenatally can generate biases or preferences for socially derived intersensory redundancy in the fetus or embryo. This "grabbing" of attention by redundant information would facilitate perceptual processing, learning, and memory for temporal and spatial features of social stimuli, thereby selectively educating attention to important and meaningful aspects of social stimulation during early development (Bahrick & Lickliter, 2012). This selective deployment of attention would in turn support the emergence of neonatal biases or preferences that would in turn further promote the development of early social responsiveness and motivation. These biases or preferences are likely critical for the development of individual recognition, social learning, as well as building the foundation for detecting meaning in speech and affect.

Our quail model provides a means to investigate this intriguing possibility. Work is currently underway exploring to what extent the amount and type of intersensory redundancy available during the late stages of prenatal development can facilitate quail neonates' social orientation, social learning, and individual recognition during early postnatal development. For example, we are testing whether the availability of redundant trimodal stimulation, which provides a greater amount and range of redundancy, can increase facilitation of attention, learning, and memory for social stimulation when compared to bimodal or unimodal exposure. Briefly, our paradigm involves providing embryos with various combinations of prenatal vestibular, auditory, and visual stimulation typically provided by the maternal hen as she leaves and returns to the nest and assessing effects on subsequent postnatal social orientation, social learning, and individual recognition of conspecifics (Vaillant, Harshaw, Jaime, Bahrick, & Lickliter, 2010).

Prenatal Roots of Contingency Detection and Contingency Learning

One key aspect of early social responsiveness is neonatal contingency detection and contingency learning. Detecting contingencies can be considered a foundational skill on which other perceptual, cognitive, and social skills develop. Tarabulsy, Tessier, and Kappas (1996) have argued that the ability to detect contingencies allows for predicting events and organizing behaviors in coherent ways, both to attain desirable outcomes and to avoid aversive consequences. Learning about cause and effect

and discovering that one's own actions can influence events provides a key basis for infants' emerging sense of social engagement and competence (Rochat, 2001; Watson, 1979). A large body of research has shown that human infants are able to learn contingencies between events that are independent of their actions as well as learn a contingency whose manifestation is dependent on their actions (e.g., Bahrick & Watson, 1985; Millar & Weir, 1992; Rochat, 2001; Rovee-Collier, 1987; Tarabulsy et al., 1996). Importantly, contingency learning relies on the ability to attend to and detect amodal (both temporal and spatial) features of stimulation.

Findings from quail neonates have likewise demonstrated that contingency detection and contingency learning is present during early postnatal development (Harshaw & Lickliter, 2007; Harshaw, Tourgeman, & Lickliter, 2008). For example, we found that postnatal presentation of an individual maternal call contingent on quail neonates' own vocalizations dramatically modifies the acquisition and maintenance of their species-typical auditory preferences in the first days following hatching (Harshaw & Lickliter, 2007). Our previous research had shown that quail embryos and chicks require up to 240 min of passive auditory exposure to an individual maternal call to subsequently remember and show a preference for that familiar call over a novel maternal call (Lickliter et al., 2002; Lickliter & Hellewell, 1992). In sharp contrast, we found that quail chicks receiving exposure to a maternal call contingent on their own vocalizations were able to learn the same maternal call following less than 5 min of total exposure and preferred that familiarized call for at least 24 h following exposure. To put these results in perspective, our previous studies using passive exposure to a maternal call required approximately 3000 repetitions of the call to foster a preference for that call over a novel maternal call. Chicks receiving contingent exposure to a maternal call required less than 45 repetitions. Whether and to what degree such contingency detection and learning skill is present prenatally is not yet known. However, evidence of prenatal interaction between parents and embryos has been documented in a number of avian species (e.g., Norton-Griffiths, 1969; Tusculescu & Griswald, 1983), suggesting the availability of socially based contingent stimulation even prior to hatching.

Interestingly, a number of studies have reported that preterm infants show deficits in contingency detection and learning when compared to full-terms (e.g., Gekoski, Fagen, & Pearlman, 1984; Haley, Grunau, Oberlander, & Weinberg, 2008; Haley, Weinberg, & Grunau, 2006), suggesting that some features of prenatal experience likely contribute to the development of these critical skills. For example, Haley et al. (2008) used a conjugate mobile reinforcement paradigm (where the overhead mobile movement is contingent on the infant's foot kicking response) and found that preterm infants differed from full-terms in their responsiveness to contingency. Preterm infants showed less evidence of learning, spent less time looking at the mobile, had lower cortisol levels, and showed greater heart rate responses to contingency when compared to full-term infants. As suggestive as these findings are, we currently do not know to what extent prenatal sensory experience contributes to the emergence and development of contingency detection and contingency learning. We are currently working with our animal model, the bobwhite quail, to identify the specific prenatal experiences that might foster (or interfere) with neonates' contingency detection and contingency learning during early postnatal development.

If our hypothesis is correct that features of normally occurring prenatal sensory experience, such as multimodal stimulation and intersensory redundancy, are critical to the emergence of contingency detection and learning, then quail embryos receiving modified prenatal sensory experience should benefit less from postnatal contingency exposure when compared to unmanipulated chicks. As a result, they would be likely to fail to remember and prefer a familiarized maternal call in the days following contingent training to that call. As a first step in exploring this research question, we prenatally exposed groups of quail embryos to either augmented prenatal auditory stimulation or unusually early visual stimulation in the days prior to hatching (Raju, Bahrick, & Lickliter, 2013a,

2013b). In the *Auditory* condition, embryos received increased amounts of auditory stimulation (tones with varying pitch) continuously for the last 2 days of prenatal development. In the *Visual* condition, embryos received unusually early visual stimulation (a light suspended 5 in. above the embryos) for 45 min each hour for the last 7 days of prenatal development. Control embryos received no supplemental prenatal sensory experience. Following hatching, all chicks from each of the three groups were individually trained at 24 h of age using a contingency exposure paradigm, in which they were presented a single burst of an individual bobwhite maternal call each time they vocalized over the course of a 5 min session. Auditory preferences for the familiarized maternal call were assessed at 48 h following hatching by means of a simultaneous choice test between the familiar call and a novel variant of the bobwhite maternal call. All chicks were tested only once. Results revealed that chicks that had received either augmented auditory experience or unusually early visual experience prior to hatching failed to benefit from their postnatal contingency training, showing no preference for either the familiar or novel maternal call during testing. In contrast, control chicks showed a significant preference for the familiarized call over the novel maternal call (Raju et al., 2013a, 2013b). These findings, while preliminary, suggest that modified prenatal sensory experience can interfere with contingency learning in quail neonates. Additional research is needed to determine what aspects of the auditory or visual exposure (timing, amount, intensity) contribute to the observed impairment in early contingency learning.

Conclusion

Prenatal experience is both a formative and regulatory force in the process of development. As a result, birth is not an adequate starting point for explanations of perceptual, cognitive, or social development. As we have briefly reviewed in this chapter, animal-based research has provided a body of evidence in support of the trans-natal continuity of neonates' emerging perceptual biases and preferences. Human based research has likewise documented such trans-natal continuity (e.g., DeCasper & Fifer, 1980; Kisilevsky et al., 2003). Simply put, it is becoming increasingly clear that young infants' biases, predispositions, and preferences are not prespecified; rather, they develop through experience (see Moore, 2009 for discussion). Neonatal preferences are shaped by prenatal experience (see Harshaw & Lickliter, 2011; Schaal et al., 1998). This insight has important implications for the study of early perceptual, cognitive, and social development and argues for the value of better integrating the prenatal period into theories of both typical and atypical development.

Shifting the focus of the study of fetal development from *whether* prenatal experience contributes to perceptual, cognitive, or social development to *how* particular experiences at particular times influence the course of early development is a key step in advancing developmental science. We still have a long way to go in realizing this ambitious goal, and the use of animal models is an important component of this challenging quest. Comparative developmental psychobiology can provide useful methods, models, and conceptual frameworks for identifying and assessing both organismic and environmental factors contributing to the emergence of specific perceptual, cognitive, and social skills. Our work with precocial bird embryos and hatchlings has found that the features and properties of available prenatal sensory stimulation (such as amount or intensity, the timing of presentation, and the sources of stimulation) coact with organismic factors (such as the stage of organization of the sensory systems and previous history with properties of stimulation) to guide and constrain perceptual differentiation, social learning, and memory. We are still a long way from fully understanding the specific pathways and processes by which prenatal sensory ecology influences perceptual, cognitive, and social development. Further research on this topic across different species, levels of analysis, and methods should be an important priority for developmental science.

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2 Fetal and Birth Experiences: Proximate Effects, Developmental Consequences, Epigenetic Legacies

April E. Ronca and Jeffrey R. Alberts

Abstract

Understanding how prenatal experience shapes function is fundamental knowledge with profound implications for developmental research and clinical approaches. In this chapter, we review four conceptual and/or mechanistic advances that have influenced our empirical studies of fetal and perinatal rats. These are: (1) *Developmental Origins of Adult Disease*, (2) *Epigenesis and the Identification of Epigenetic Mechanisms*, (3) *Gottlieb's Articulation of Experience as Mechanism*, and (4) *Ontogenetic Adaptation*. We describe our analyses showing that sensory stimulation in utero and during birth help the newborn achieve important postpartum milestones. Our psychobiological approach is 'ecologically' oriented which, for fetal research, implies consideration of the adaptive significance of behavior within the uterine habitat. Thus, we made detailed quantifications of natural intrauterine stimuli arising from the mother and the environment of the womb and then modeled these sensory events in the laboratory. Our findings indicate that, during pregnancy and parturition, the mother's behavior and physiology generate copious episodes of stimulation that are *detectable by the fetus* and *alter sensory function*. The absence of these natural forms of sensory input can impede the perinatal expression of adaptive responses, including vital postpartum milestones (i.e., pulmonary respiration and sucking responses). Further, we identified perinatal catecholamine release as a putative mechanism underlying these effects. These new views of proximate, long-term, and potential epigenetic effects of intrauterine and birth stimulation in an animal model can contribute to improving human clinical outcomes during postpartum life and beyond.

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Introduction

Publication of *Fetal Development: A Psychobiological Perspective* (Lecanuet, Fifer, Krasnegor, & Smotherman, 1995) marked a new awareness among behavioral scientists about the nature of the uterus as an environment and the fetus as its inhabitant. No longer was the womb a silent, placid refuge. Instead, the intrauterine environment was seen as dynamic, complex, and stimulating. A late-term, mammalian fetus was increasingly appreciated as a sentient being. Reflecting on the first 20 years of "systematic fetal behavior investigation", Lecanuet and Krasnegor (1995) regaled in a view of an exciting scientific landscape. An important feature was that the fetus experiences intrauterine stimuli (Lecanuet et al., 1995; Smotherman & Robinson, 1988).

At the time, it was well recognized that early experience is important. Until then, however, "early experience" meant *postnatal* experience. Accumulation of data and ideas, along with some technical innovations, had enriched the experimenters' view of the fetal world and through this lens was envisaged a new, 'fetal psychobiology'. Through this lens it also was seen that the pregnant mother contributes to fetal development in ways that extend beyond the traditional role of a walking incubator plumbed for fetal nutrition and waste removal.

Like natural landscapes, scientific landscapes continuously change. Indeed, in the past 20 years there have been upheavals, levelings, successions, and blossoming across the scientific terrain on which grows our understanding of fetal life. We briefly discuss, below, a set of ideas and findings that have influenced our empirical studies and their interpretation. All four of these areas, we believe, have contributed to fetal and perinatal sciences and are worthy of general recognition here.

Early Developmental Origins of Adult Disease

In recent years, there has been an explosion of research on prenatal determinants of adult health and disease. Much of this work was inspired by epidemiological analyses by Barker (1995) in which they reported that birth weight (a readily available measure of fetal growth) can predict later health status. Prominent in the early studies were findings derived from human catastrophes such as the Dutch Famine (1944–1945), when food intake was a mere 400–800 kcal per day (cf., the adult daily requirement of 1500–2000 kcal). Women who were pregnant during this famine delivered babies with lower than normal weights and the morbidities and mortalities in the adult offspring were eventually recognized and investigated widely (see Barker & Thornburg, 2013). Specifically, portions of the population that endured fetal growth attenuation but then "compensated" postnatally and attained average height and weight in childhood nonetheless developed serious morbidities such as coronary heart disease and type 2 diabetes (Barker & Osmond, 1986; Phillips, 1996). The risk of developing these diseases was a function of the severity of the prenatal growth condition.

Human epidemiological studies, combined with cellular, biochemical, and animal research, led to the general concept of 'fetal programming'—the idea that intrauterine events set the stage for disease states in adulthood (Barker & Thornburg 2013). As the purview of these analyses expanded to include early postnatal influences, the terms 'Fetal', 'Prenatal', or 'Intrauterine' Programming migrated toward the more encompassing, 'Developmental Programming'. Collectively, the data support the view that various alterations in the early environment can have enduring consequences for metabolic,

cardiovascular, and neuroendocrine pathophysiology across the lifespan that persists across generations (Glover, O'Connor, & O'Donnell, 2010; Meaney, Szyf, & Seckl, 2007; Nijland, Ford, & Nathanielsz, 2008). Stress, parental care, and nutritional status (and relatedly, gut microbiota) are some of the early influences that have been shown to enhance susceptibility to a broad range of physiological and mental disorders in later life (Arrieta, Stiemsma, Amenyogbe, Brown, & Finlay, 2014; Babenko, Kovalchuck, & Metz, 2014; Brunton, 2013; Meaney et al., 2007; Seckl & Meaney, 2004, 2006). Further, there is a growing appreciation that these programming effects are sex-specific, affecting males and females differently (Bale, 2011; Brunton & Russell, 2010; Brunton et al., 2013).

The dramatic phenomena and associated mechanisms constituting this field instantiate the idea that prenatal deficits that appear rectified during early postnatal life, can nonetheless reappear lawfully later in life as altered function. The timed appearance of seemingly remote *consequences* was buoyed by invoking the metaphor of "programming", but it is important to recognize that unlike a true 'program' in which specific information is encoded and 'read out' faithfully, the actual mechanisms associated with prenatal influences exist as general, uninformed, probabilistic alterations in low-level molecular events. These important perturbations are expressed through webs of complexity, rather than as a reading of instructions with isomorphic expressions. The challenge has been, and remains, to identify and understand the intervening mechanisms. Some remarkable progress has been made.

Epigenesis and the Identification of Epigenetic Mechanisms

The term 'epigenetic' was coined in the 1940s by Conrad Waddington (1942). As an embryologist, Waddington studied cell differentiation and the subsequent maintenance of cellular identity; he believed that a cell's position in the developing body—the cell's environment—determined its differentiation and identity. To illustrate the idea that genes produce a phenotype through interactions with the cellular environment, and to illustrate how early events can direct or canalize the course of development, Waddington (1957) created the visual metaphor of a marble rolling down an 'epigenetic landscape' over which the path of the marble (developmental process) is guided (canalized) through different, more differentiated valleys along a slope (Fig. 2.1, left).

Waddington's epigenetic landscape provided a useful and influential framework but it predated knowledge of the structure of DNA, so it remained a conceptual tool awaiting connection to mechanism. And, such connections have been made! One area of great advancement is the elucidation of DNA methylation, a molecular process by which the ability of specific genes to engage in protein expression is deactivated. Such methylation involves a methyl group attaching to nucleotides in a gene's promoter, where it binds a repressor protein, preventing expression. Other molecular mechanisms can release protein expression. Thus, through methylation and other newly recognized processes such as histone modification, chromatin remodeling, and microRNAs (miRNAs), inputs from the environment can lawfully modulate gene activity across generations, without altering the composition of the genome itself. Hence the label, epigenetic.

Goldberg, Allis, and Bernstein (2007) revised and updated Waddington's epigenetic landscape, incorporating a suite of now known epigenetic mechanisms. The new epigenetic landscape is modeled after a pinball machine (Fig. 2.1, right). As the pinball traverses a complex and dynamic landscape, there is a concatenation of molecular events through which stable and heritable changes in gene expression and phenotype occur without alteration of the DNA sequences.

Importantly, epigenetic patterns induced by environmental stimuli (including the social environment) can be maintained across generations. In other words, they are heritable—yet the genome is unchanged. These epigenetic effects thus provide a mechanism for the kinds of transgenerational, non-Mendelian inheritances that

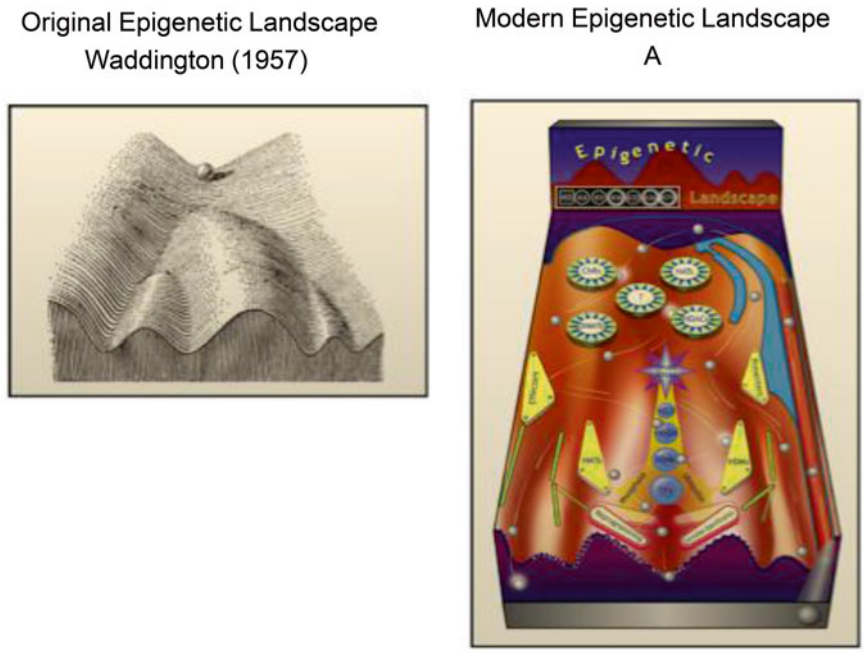


Fig. 2.1 (*Left*). Waddington's epigenetic landscape. The *ball* represents some tissue or behavior at an early developmental timepoint. Development progresses as the *ball* descends through the landscape. The mechanisms regulating the development process are represented by the positions and shapes of the contours. (Reprinted with permission from Waddington, 1957.) (*Right*). Modern epigenetic landscape. chromatin, the complex of DNA and its intimately associated proteins, shapes the features of a cell's epigenetic landscape. Histones are the chief protein components of chromatin that act as spools around which DNA winds, playing an important role in gene regulation. A variety of factors now known to regulate epigenetic phenomena (Effector and Presenter proteins) involved in histone modifications, histone-modifying enzymes, ChR-Chromatin remodelers, DNA methyltransferases, and other players in this dynamic epigenetic game are shown directing the complex movement of pinballs (cells) across Waddington's landscape. (Reprinted with permission from Goldberg et al., 2007)

have long been reported by psychobiologists studying multigenerational consequences of early stress (e.g., Champagne & Meaney, 2001; Denenberg & Rosenberg, 1967; Francis, Diorio, Liu, & Meaney, 1999; Levine, 1967). This exciting research domain now combines investigations of prenatal and early postnatal influences, molecular and behavioral mechanisms, and developmental-evolutionary processes.

The metaphors and mechanisms of epigenetics have magnified our views of perinatal life and are having profound effects on research and clinical approaches. These mechanisms may pertain directly to understanding the effects of prenatal stress on fetuses and their subsequent development. Likewise, early phases of maternal care have been linked to methylation patterns and to the nongenetic, transgenerational inheritance of stress responsivity as well as specific behavior patterns (e.g., Brunton & Russell, 2010; Champagne & Meaney, 2001; Liu et al., 1997; Weaver et al., 2004).

Gottlieb's Articulation of Experience as Mechanism

The importance in developmental thinking of the term, *experience*, is reflected in the frequency of its usage, including the common phrase, "early experience". Yet, the term has long defied precise and practical definition. The behavioral embryologist, Gilbert Gottlieb, elevated the stature of experience in ontogenetic processes by articulating the various levels on which it can occur (Gottlieb, 1973). In his formulations, Gottlieb built on several predecessors whom he admired. Like Kuo (1967) and

Schneirla (1957), he was highly inclusive and saw experience occurring and acting on a multitude of levels, from single neurons to entire multimodal perceptual systems and actions. In fact, Gottlieb equated basic, provocative forms of cellular activity, or *stimulation* with the cellular or system *function* that is evoked, as well as more integrated sensory-perceptual processes that are commonly treated as *experience*.

There followed his articulation of "the roles of experience in early development" (Gottlieb, 1976) in which he posited that experience (on any of the numerous levels he recognized) can play a general, on-going role of *maintenance* of developmental process. Alternatively, experience can also work on development through *facilitation*, which basically refers to rate regulation. The third role of experience in Gottlieb's systems view is *induction*, a process by which development is directed down one of more or less specific, alternative pathways (resembling, of course, the lower valleys of Waddington's landscape), resulting for example, in species-specific perceptual preferences (e.g., Alberts & Brunjes, 1978; Gottlieb, 1971a).

Gottlieb's formulation of the "roles" of experience translates directly into a definition of *experience as class of mechanism*. The instantiation of experience as a definable, set of mechanisms that contribute to developmental process, rate, and differentiation was an enormous contribution [see also Aslin (1981) who further subdivided different types of experiential mechanism]. The basic construct and the formulation of each type of mechanism has, we think, gradually and importantly permeated developmental thinking and the shape of research programs. In contrast to the explosive impact of findings in the areas of epigenetics and developmental programming, described above, influence on research of the roles of experience has gradually shaped the field, but the influence has been palpable.

It should be noted that Gottlieb's thinking was likely shaped by his prior scholarly focus on the comparative development of sensory systems, as summarized in his magnificent, integrative review (Gottlieb, 1971b) and embodied in his original research on the development of species-typical perceptual preferences in ducks (Gottlieb, 1971a). Thus, it is understandable that although his conceptual formulation is applicable to numerous levels of development (neuronal, neural systems, neuroendocrine, motor, and more), Gottlieb emphasized how stimulation/experience affect the development of sensory systems and behavior. It is significant that Gottlieb studied prenatal perceptual development. Thus, his ideas and their formulation are especially pertinent to questions of fetal development and behavior. We expect his influence in this area will only increase in years to come.

Ontogenetic Adaptation

The construct of ontogenetic adaptation has lived in relative dormancy until recent years as its influence on perinatal research as well as on clinical practice has become increasingly discernible. The rise of ontogenetic adaptation may have begun with keen and counterintuitive insights from neuroembryology. Oppenheim (1981, 1982), in particular, contrasted the developmental processes of progressive building of the nervous system with formative, regressive processes of retraction and loss during development. The behavioral concomitants of this perspective highlighted attributes that come and go, and sometimes reappear (Alberts & Harshaw, 2014). Conceptually, ontogenetic adaptation is rooted, in part, in evolutionary biology, which contributed the concept of adaptation as an historical process whereby the forces of natural selection shape and preserve organismal features. Ontogenetic adaptation is also connected to the traditions of physiological ecology that illuminate how specific features of an organism serve proximate ends and functions. Finally, ontogenetic adaptation is also an overall perspective on development that provides a framework within which ontogeny comprises a sequence of adaptations (of physiology and behavior, as served by all the underlying systems and processes) to a corresponding sequence of developmental contexts.

Using ontogenetic adaptation as an overall perspective, we can see the mammalian fetus as

an organism that exhibits a suite of specializations and refinements (i.e., adaptations) for life in a "ecological habitat" that is occupied by all mammalian fetuses during prenatal life (early developmental habitats of other genera, e.g., birds, reptiles, and insects, are similarly characteristic within groups but are vastly different than the mammalian type). Despite numerous species differences, the uterus is a universal habitat for fetal stages of mammalian development. It is in this uniquely mammalian world that a mammalian fetus first 'earns its living'. Vital functions, such as respiration, nutrient intake, and waste excretion are accomplished with specializations such as an umbilical cord and placenta.

With the event of birth, there is a near-instantaneous change in habitat. The uterine environment is replaced by the 'outside' world, including the exterior of the mother's body, and basic organismal functions are immediately accomplished with a new set of specializations—pulmonary respiration, oral intake, and intestinal processing and excretion. Thus the newborn exhibits a corresponding suite of adaptive specializations: Processes to clear fluids from the lungs, enabling gas exchange, and a reordered cardiovascular system for rerouting the blood—all in the service of respiration and regulation of blood gases; a total organization of sensory-perceptual orientation that brings the newborn's perioral areas into contact with a nipple and enables oral grasping, suckling, swallowing, and coordinated breathing; the onset of thermogenesis and body temperature regulation; orientations and movements defined by gravitational forces, and many other new habitat-related functions. There are corresponding adaptive specializations for these functions and for those that follow, as the mammalian newborn develops beyond its newborn niche and, for example, enters the phase when mother's milk is replaced by solid food, which is acquired, manipulated, digested, and absorbed differently. The interested reader can find discussions of ontogenetic adaptation in a variety of sources (e.g., Alberts & Cramer, 1988; Alberts & Harshaw, 2014; Baldwin, 1896; Blumberg, 2005; Galef, 1981; Hall & Oppenheim, 1987; Oppenheim, 1981).

Ontogenetic adaptation now pervades fetal and perinatal psychobiology. Its influence is sometimes explicit, but often implicit and can be seen in a variety of insights into mechanisms that are best framed as ontogenetic adaptations and approaches to developmental stages that are informed by inclusion of contextual and organismal variables.

Model Rats and Rat Models

Our research on fetal behavior was conducted with laboratory rats (*Rattus norvegicus*). Often referred to as *the* rat, the Norway rat is but one of more than a thousand species of murid rodents. Norway rats are distinct among their rodent brethren as outstanding generalists. They can thrive in fields, barns, burrows, basements, attics, dumps, ships, and laboratory cages; we have worked with mothers and fetuses that flew successfully in spacecraft! Most rodent species live within restricted ecological settings and tend to specialize in a narrow range of foods. The adaptability and omnivory of R. norvegicus underlies its status as a perennial pest in human settings as well as its value as a domesticated biomedical subject.

Rodents give birth to multiple offspring that, in all but a few species, are delivered at an early stage of development. They are *altricial*, born with immature sensory systems (e.g., sealed eyes and ears), limited motor repertoire, and are fully dependent on the mother for nutrition, heat, and protection.

Although Norway rats are not *the* rat, phylogenetically speaking, researchers have gleaned from them a large body of general and generalizable knowledge. This has been accomplished by focusing on traits that are common across related species. The basis of shared features is believed to be common ancestry, so that species that exhibit common traits do so because the trait has been retained from a common ancestor. Such retained traits are thus considered to be evolutionarily *conserved*. Our interests in the rat are not to describe a miniature human replica or to model directly the human condition. Rather, we seek general biological principles of mammalian development and, as such, there will be relevance to humans as mammals. Even more important, perhaps, this

adaptive, ecological framework provides methodological and conceptual insights for clinical inquiries of the human fetus and newborn.

A conserved trait that has been important to our research is onset of function in sensory systems. This is a developmental trait. As mentioned earlier, Gottlieb (1971b) and Alberts (1984) have discussed evidence that sensory function begins in a specific order. The most thoroughly documented sequence is tactile → vestibular → auditory → visual, with the chemical senses probably following tactile, and thermal also occupying one of the primary places in the order (see Alberts, 1984). Note that this sequence refers to the initial responsivity of each sensory system, not to the attainment of full function, maximal sensitivity, or to perceptual capabilities such as acuteness or discrimination. There exists a dissociation between onset of function and attainment of full function (see Alberts, 1984).

Recent research has revealed important ways in which the activity of one sensory system can affect the development of another. Thus stimulation of the embryonic visual system can alter the development of audition (see Chap. 1). In this way, it is clear that, the order of onset of sensory function structures the sequence of experience of the organism and provides a natural 'scaffold' by which sensory experience can regulate development within and between different systems. Thus, conservation of the onset of sensory function provides a general structure to the development of sensory function and behavior for all mammals and, indeed, all vertebrate species that have been analyzed in this manner.

The Mammalian Experience of Intrauterine Life and Birth

In the body of this chapter that follows, we apply aspects of the four scientific traditions briefly outlined to a sequence of investigations that we conducted with rats. We will treat the mammalian uterus as an environment encased in the behaving body of a mother. This leads directly to the challenge of characterizing that environment. Because the purpose of our inquiry is to understand the experience of a fetus residing in a uterine environment, we evaluate fetal sensory function in relation to the kinds and levels of stimuli therein. The presence of stimuli in the uterus does not ensure that an immaturely formed fetus will detect such stimuli or respond to it. Evidence that fetuses experience maternally derived stimuli in utero (and during parturition) leads naturally to questions about the consequences of such experience. As we uncover the different kinds of consequences that derive from intrauterine and birth stimulation, we get a new view of both proximate, long-term, and potential epigenetic effects of early mammalian experiences.

Fetal Experience in Relation to Intrauterine Stimuli

Throughout gestation, the mother and her offspring are exquisitely intertwined, forming an integrated, biological system within which the mother, gestating siblings, and fetus itself each contribute in significant and meaningful ways to the fetus's sensory milieu (Alberts & Cramer, 1988; Alberts & Ronca, 1993; Ronca & Alberts, 1995a). The mother's behavior and physiological functions, combined with sibling movements, self-stimulation, the fluid-filled amniotic sac, uterine contractions, and thermal flux during parturition (Alberts & Cramer, 1988; Ronca & Alberts, 1995a; Ronca, Kamm, Alberts, & Thelen, 1994; Ronca, Lamkin, & Alberts, 1993) create distinct and quantifiable sources of prenatal epigenetic experience (Ronca & Alberts, 1994, 1995a, 1995b, 1995c).

We have described and quantified the prenatal sensory environment of the rat from the fetus' perspective. Using videographic analyses, we analyzed the dam's activities during gestation, labor, and delivery. Our approach and quantitative analysis is illustrated in Fig. 2.2. Pregnant females remain surprisingly active throughout the final week of gestation, in fact, as active as nonpregnant females. Exploratory movements, feeding, drinking, self-grooming, and other activities of the rat dam pitch, turn, accelerate, and expose fetuses to

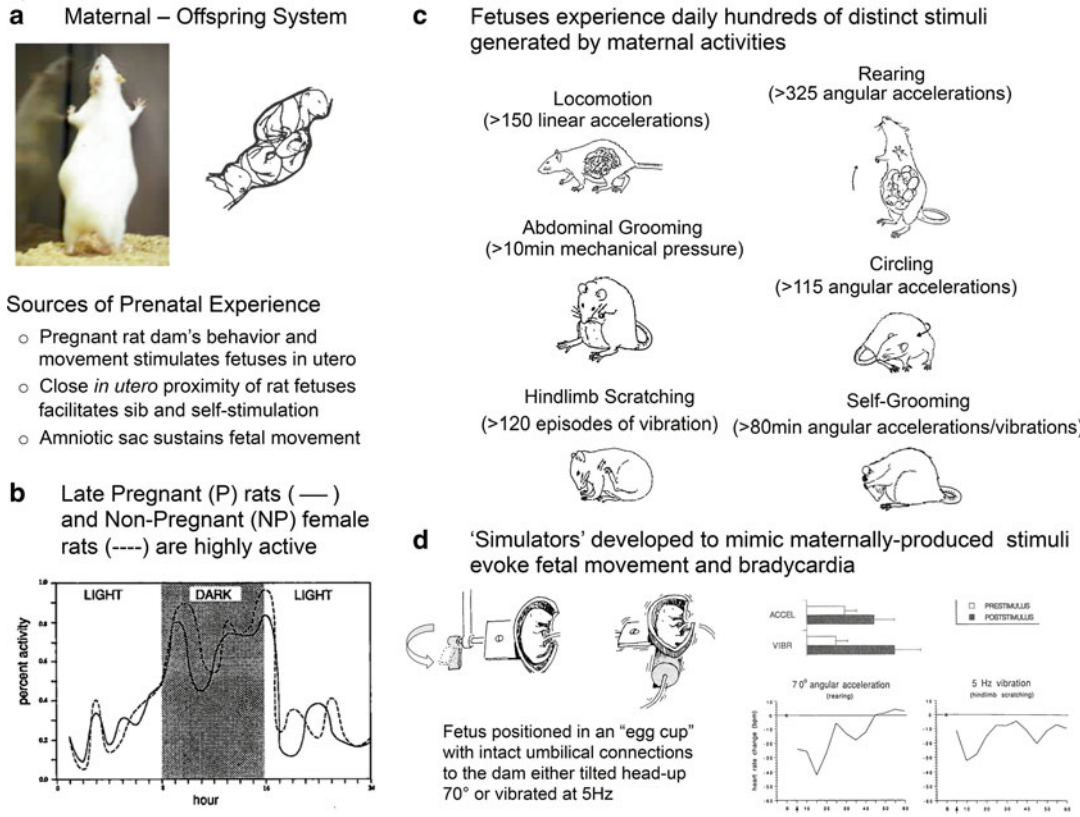


Fig. 2.2 Quantitative and qualitative videographic analysis of naturally occurring sources of prenatal epigenetic influence. (**a**) Mother and offspring form a functional biological system. Maternal, intersibling, and fetal self-stimulation occur in utero. The amniotic sac shapes temporal patterning of movement. (Adapted from Alberts & Cramer, 1988.) (**b**) Overall activity levels of Pregnant (P) and Nonpregnant (NP) rats across light and dark phases of the cycle on Gestational (G) day 20 and 21 of the rat's 22-day pregnancy. (Adapted from Ronca et al., 1993.) (**c**) Specific maternal activities quantified on G15, 18, and 21 showing minimum frequency or duration of each behavior per 24 h period. (Adapted from Ronca et al., 1993.) (**d**) 'Simulators' developed to mimic maternal stimuli. Dams received a spinal blockade to eliminate movement and sensation below the ribcage, and her lower body maintained in a 37.5 ± 0.5 °C buffered saline bath. For testing, the fetus was placed in an "egg cup" that was attached to a microscope stand, permitting fine vertical control over the position of the fetus in the water bath, and preventing tension from being applied to the umbilical cord. This configuration stabilized the fetus, enabling controlled, reliable application of simulated maternal stimuli. From *left* to *right*: Hindlimb scratching (5 Hz vibration); Rearing (angular acceleration via 70° head up tilt). Stimuli were 5 s in duration. Data (*top right image*) show the average prestimulus (30 s) and poststimulus (30 s) movement of G21 fetuses to angular acceleration (*top bar pair*) and vibration (*lower bar pair*). The *images* in the *lower right* show average cardiac response of G21 fetuses to angular acceleration (*lower left graph*) and vibration (*lower right graph*) expressed as change from prestimulus baseline heart rate and plotted for 60 s poststimulus. Robust behavioral and bradycardiac responses were observed to each stimulus type. (Modified from Ronca & Alberts, 1994)

mechanical pressures (Ronca et al., 1993). Fetuses are exposed to linear acceleration as the dam ambulates, mechanical pressure as she grooms her abdomen, vibration as she self-grooms her head and body, and hindlimb scratches, these latter actions involving rapid stereotyped and repetitive head and limb movements. Fetuses are exposed to numerous episodes of angular acceleration as the dam rears, circles, and self-grooms. To the extent that maternal activities transmit to fetuses sensory experiences of acceleration, pressure, and vibration, our results indicate that the fetus in utero is exposed to hundreds of specific and patterned stimuli each day, even within 24 h of birth.

In this analysis, we were able to make highly detailed quantifications of each maternally generated stimulus that we identified. For example, from frame-by-frame analysis during maternal rearing, fetuses in utero travel an average distance of 63°(±3°) or 1.10 rad (±0.05 rad), at an angular velocity equal to 0.56 rad/s. Dams retained an upright posture for 1–18 s. These values enable general estimates of fetal excursion during rearing episodes; the precise amount of stimulation will vary as a function of uterine position. Recall that the rat is a polytocous species, bearing multiple offspring that populate various distinct positions along the uterus. During an episode of maternal rearing (Fig. 2.2), fetuses residing at the ovarian end of a uterine horn would travel the greatest distance and at the greatest velocity, whereas fetuses at progressively more caudal sites would make smaller and slower excursions. In humans, unborn babies toss and turn, assuming many different positions in the womb across gestation (e.g., transverse, head-down, posterior, breech). For both singletons and multiples, this constant flux poses distinct experiential and developmental implications for sensory, neural, and physiologic systems (e.g., Previc, 1991). Uterine position may be an important yet grossly underappreciated determinant of fetal experience and epigenetic variation.

Both intrauterine and extrauterine stimuli likely affect fetuses differently at different times during gestation, as various sensory systems begin to function and undergo early phases of maturation. In addition, the fetal microenvironment changes across gestation. Amniotic fluid volume declines during late gestation as the fetus continues to grow and expand within the amniotic sac and uterus (Smotherman & Robinson, 1988). The net effect is that, as birth approaches, the fetus is less buffered from mechanical and other forms of stimulation. The pregnant dam's behavior and movements may provide the most direct and intense stimulation to fetuses at this time. On the final observation day of our study (G20-21), when amniotic fluid is nearly exhausted, fetuses are exposed to hundreds of cutaneous and vestibular stimuli. Systematic changes in the uterine microenvironment over the course of gestation may help regulate or modulate the effects of experience and corresponding epigenetic alterations in the developing fetus.

Varieties of Fetal Experience

Understanding the intrauterine environment and prenatal sensory competence are essential for ascertaining the extent to which available stimuli can be transduced by fetal sensory systems and the consequences of such stimulation. After measuring various elements of the perinatal environment and creating procedures to deliver appropriately calibrated cues, we tested the sensory abilities of rat fetuses to detect stimuli derived from maternal activities. Figure 2.2 shows that these included tilts that mimic maternal rearing, and vibration that match intrauterine events created when a pregnant dam grooms herself. Each form of calibrated stimulation evoked robust behavioral and cardiac responses from perinatal rats (Ronca & Alberts, 1994). The fetus's HR and behavioral responses demonstrated transduction of stimuli normally encountered during fetal life, and showed that functional linkages to efferent systems are in place prior to birth.

Collectively, these results provide direct evidence for fetal experience as a mechanism that can regulate developmental processes. Formative effects of stimulation on the development of sensory systems and behavior are well documented and there is now ample evidence that prenatal (or prehatching) sensory experience can influence perceptual development (Kenny & Turkewitz, 1986; Chap. 1).

In addition to its effects on sensory-perceptual development, early sensory function also can affect behavioral as well as anatomical development. When a fetus detects a change in its environment, behavior is activated. The importance of activating fetal behavior is emphasized by findings that reductions in embryonic limb movements, even for a relatively brief period, can permanently block the joint flexibility—that is, limb movements are part of the formative process of the musculoskeletal apparatus (Drachman & Sokolov, 1966; Moessinger, 1983). Similarly, manipulations that reduce fetal swallowing or early respiratory movements have been directly associated with impairments in gastrointestinal and pulmonary development (Liggins, 1982). The present results, along with those of several other studies (e.g., Ronca et al., 1994; Smotherman &

Robinson, 1988), demonstrate that fetal sensations instigate fetal movements, thus suggesting that the onset or maintenance of developmentally adaptive movements are regulated by fetal experiences.

Omitting and Enhancing Fetal Sensory Experience

Manipulation of sensory input during ontogenesis is a time-honored approach to investigating development of the senses. Reducing input by dark-rearing (Hubel & Wiesel, 1982), plugging the ears (Batkin, Groth, Watson, & Ansberry, 1970), obstructing the nares (Brunjes, 1994; Kucharsky & Hall, 1987), eliminating tactile input supplied by the vibrissae (Woolsey & Wann, 1976), or augmenting sensory experience by using imposing sounds, light, or odorants (Gottlieb, 1971a, 1971b; Todrank, Heth, & Restrepo, 2011; Chap. 1) have yielded important insights into how stimulation plays active, formative role(s) during ontogenesis in the development of neural architecture and behavioral function.

Sensory deprivation during early life produces enduring deficits in sensory function. For example, clinical observations (Peters, Litovsky, Parkinson, & Lake, 2007; Rubinstein & Miller, 1999) have shown that proper acoustic input is critical for the development of hearing; delayed provision of a cochlear implant in children can prevent acquisition of proper understanding of spoken words. Similarly, understanding the roles of early stimulation has augmented clinical approaches in the treatment of visual impairments (Maurer, Lewis, Brent, & Levin, 1999).

Too much remains unknown about fetal behavior; the lack of basic information can be acutely felt in the NICU, where there reside many infants developing in an environment markedly different than a stage-appropriate uterus. Practitioners dedicated to "developmental care" seek to provide stage-appropriate support to prematurely born infants, but much of these efforts are guided by intuition or guess work. What is the daily frequency of swallowing by a 34-week fetus? How does this compare to the swallowing rate of an age-matched premie in an incubator? Perhaps some of the feeding difficulties typical of prematurely born infants are related to derailing of early swallowing when they are displaced from the womb and thus the experiences of provoked swallowing of amniotic fluid. Interestingly, Lau and Smith (2012) intervened with randomized groups of very low birthweight infants that were treated with regimes of sucking exercises with a pacifier, swallowing exercises in which a small liquid bolus (0.05–0.2 ml of milk) was placed on the rear of the tongue to induce a swallow, or no intervention. The outcome measures were days from start of feeding (SoF) to independent oral feeding (IOF) and measures of rates and volume transferred. Outcomes for sucking exercise and control groups were similar, whereas infants with augmented swallowing experiences achieved IOF more rapidly—about 6 days sooner than controls; transfer metrics were similarly potentiated.

Pregnant Rats in Space: A Glimpse at Gravitational Stimulation and Fetal Vestibular Development

In contrast to other sensory systems, our understanding of neurovestibular ontogeny has progressed slowly, due, in part, to the historic difficulty of depriving young organisms of the Earth-constant and omnipresent stimulus of gravity. A growing number of techniques are proving their utility for depriving the immature mammalian vestibular system of sensory input. For example, the semicircular canals (Geisler & Gramsbergen, 1998; Geisler, IJkema-Paassen, Westerga, & Gramsbergen, 2000) of young rats have been plugged as early as the fifth postnatal day. Genetically altered mouse strains that fail to develop otoconia (*tlt*; tilted) may nevertheless compensate for much of this loss (Crapon de Caprona, Beisel, Nichols, & Fritzsch, 2004). Other mutants show depletions of sensory cells in both otoconia and semicircular canals (*Hmx2 and Hmx3*, members of the *Hmx* homeobox gene family), or other selective losses within the vestibular end organs (Fritzsch, Pauley, & Beisel,

2006) are yielding new understandings of the development and evolution of the vertebrate vestibular system, as its cellular and molecular bases are delineated (cf. Beisel, Wang-Lundberg, Maklad, & Fritzsch, 2005). Relatively little work has focused on the emergence of vestibular function. Yet within the sequentially fixed, highly conserved pattern of sensory ontogenesis in birds and mammals, vestibular function is one of the first modalities to emerge (Fritzsch, 1998; Gottlieb, 1971a, 1971b). Onset but not final maturation of vestibular function always occurs before birth or hatching (Alberts, 1984). Thus, responses to vestibular perturbation are first observed prenatally (Gottlieb, 1971a, 1971b; Ronca & Alberts, 1994; Fig. 2.2), after the neurovestibular circuitry is largely in place, albeit while still developing. As evidenced by observations of prenatal responses to angular acceleration (Gottlieb, 1971a, 1971b; Ronca & Alberts, 1994), the neural substrate, although immature, is clearly capable of transducing vestibular input.

We had the opportunity to expose prenatal rats to microgravity during the latter half of gestation, when—in the presence of Earth-normal gravitational stimulation—neurovestibular connectivity and onset of vestibular function have been dated (Ronca, Fritzsch, Bruce, & Alberts, 2008). The opportunity to examine the effects of attenuated gravitational stimulation on the onset and early development of vestibular function came with the scientific novelty of launching 10 pregnant rats in the Space Shuttle where they would spend 11 days in orbital spaceflight.

By way of background: Receptors within the inner ear, the otoconia (macule and saccule) respond to gravitational stimuli (i.e., linear acceleration), whereas receptors in the semicircular canals respond to velocity of head rotation (angular acceleration). Microgravity exposure would eliminate or blunt the gravitational stimulus (linear acceleration) but would leave available stimuli of angular accelerations. Depending on the movements of the mother's body and fetal movements during microgravity, angular accelerations could be greater or lesser than those during gestation on earth. In any event, it was likely that gestation during orbital spaceflight would differentially stimulate these two major subdivisions of the mammalian vestibular system.

We tested the hypothesis that gestation in microgravity would deprive the developing fetuses of gravistatic input to the otoconia, yet augment their exposure to angular acceleration due to altered maternal activity during weightlessness. Rats in a Space Shuttle habitat (a wire-walled cage in which they are group housed) are active and, in the weightlessness of space, the dam's behavioral repertoire would be altered. Specifically, rats in this space habitat might (and did) remain "thigmotactic" (oriented preferentially to surfaces and thus treated all the walls and surfaces as 'floor' on Earth. As the weightless dams floated and traversed the walls and surfaces of their enclosure, they turned and rotated their bodies more frequently and in orientations that were uncommon in 1-g, ground control dams, similarly housed on Earth (see Fig. 2.3).

We knew that the dam's behavior is significant because her body movements create numerous episodes of angular acceleration that can be detected by fetuses and evoke responses from them (Ronca et al., 1993; Ronca & Alberts, 1994; Fig. 2.2). It was reasonable to predict neurovestibular and behavioral changes in offspring secondary to the pregnant dams' behavior in microgravity. We used inflight videorecordings of the flight dams and corresponding videos of the synchronous (ground) controls to evaluate maternally instigated sensory input to prenatal semicircular canals. We further predicted correlated changes in function and neuroanatomical projections from the vestibular end organs. In other words, we expected a postflight phenotype comprising reduced sensitivity to gravistatic input with a corresponding reduction in projections from gravistatic receptors and increased sensitivity to angular acceleration with increased projections from semicircular canals.

Our predictions were realized: Behaviorally derived stimulation from maternal movements appears to be a determinant of vestibular sensory development. Kinematic analyses of the dams' on-orbit behavior (Fig. 2.3) suggest that, although the fetal otolith organs are unloaded in

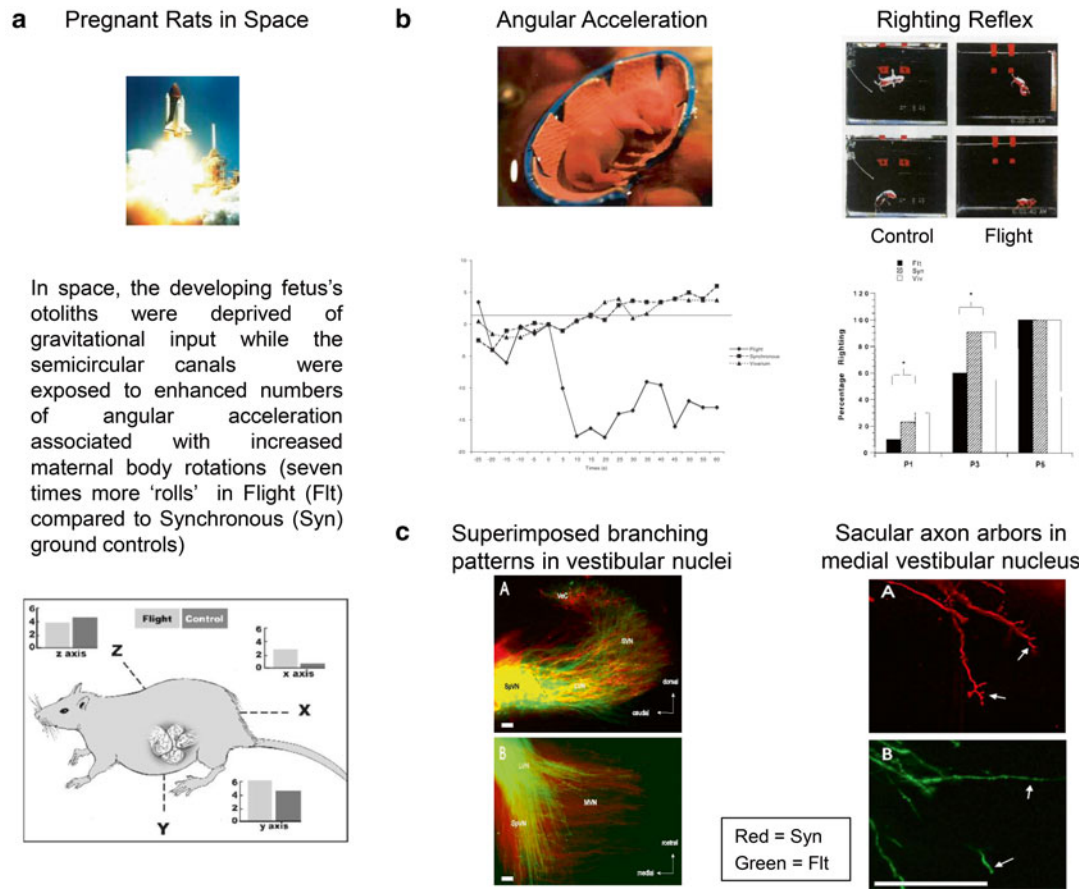


Fig. 2.3 Behaviorally derived stimulation from the mother's movement shapes vestibular system development and function. (**a**) *Upper image*: NASA Space Shuttle. *Lower image*: Relative rates of transitions of body movements over three vectors (x, y, z) shown by rat dams (flight and synchronously housed controls). Numbers of changes in each vector are expressed in degrees (angle) per second (time) as coded from inflight videorecordings. (**b**) Postflight behavioral responses. *Upper left*: G20 fetuses exposed to angular acceleration immediately postflight. Average heart rate response of Gestational Day 20 fetuses in flight ($n=4$), synchronous control ($n=3$) and vivarium control ($n=3$) conditions to the 10-s 70° head-up tilt. Heart rate (beats/min [bpm]) is plotted as the difference from prestimulus baseline (time = 0 s). The *horizontal axis* (time = 0 s) corresponds to stimulus onset. *Upper right*: Percentage of P1, P3, and P5 pups in the Flight (Flt), Synchronous (Syn), and Vivarium (Viv) conditions that achieved the prone position during vestibular righting from a supine posture in the water-immersion test. $*P<0.001$. (c) *Left images*: Superimposed branching patterns of posterior vertical canal (*A*) and saccular (*B*) axons in the vestibular nuclei of fetuses in flight (*green*) and control (*red*) conditions. (*A*) Parasagittal sections show afferent axons from the posterior vertical canal project to overlapping vestibular areas in both control and microgravity-exposed fetuses. (*B*) Horizontal sections demonstrate that afferent axons from the sacculus project more medially in control than microgravity-exposed fetuses. *LVN* lateral vestibular nucleus, *MVN* medial vestibular nucleus, *SpVN* spinovestibular nucleus, *SVN* superior vestibular nucleus, *VeC* vestibulocerebellum. Bar = 100 μm. *Right Images*: Saccular axon arbors in the medial vestibular nuclei of control animals (*top*) have more short side branches than those of flight animals (*bottom*), indicating that the development of flight gravistatic receptor afferents is delayed compared with those of controls. Bar = 100 μm. (Modified from Ronca, Fritzsch et al., 2008)

microgravity, the fetus' semicircular canals receive high levels of stimulation during longitudinal rotations of the mother's weightless body. Postflight testing revealed (a) cardiac deceleration (bradycardia) to 70° head-up roll, (b) delayed onset of body righting responses, (c) decreased branching of gravistatic afferent axons, but (d) no change in branching of angular acceleration receptor projections with comparable synaptogenesis of the medial vestibular

nucleus in flight relative to control fetuses. This latter finding suggests that, for in-flight fetuses, other vestibular afferents, such as those transducing angular acceleration, may have compensated by increasing synaptic number.

Further Reflections on Prenatal Development of the Vestibular System and Some Clinical Implications

Our findings from these spaceflight studies provide an enticing example of how the Earth's gravity may have shaped neurobehavioral and physiological adaptations in mammals. Other, related ideas now have additional cache: For example, asymmetric neurodevelopmental influences occurring in the prenatal environment have been suggested to form the basis for cerebral lateralization in humans (Previc, 1991). Vestibular lateralization can be traced to the asymmetric positioning of the fetus in utero during the final trimester that, in combination with maternal locomotor patterns, creates a lateralized shearing favoring the development of the left otolith and its neural pathways. The left otolithic advantage in most humans has been suggested to underlie reliance of the left side of the body for postural control and the right side for voluntary motor behavior, as well as the right hemisphere's specialization for most visuospatial operations. Interestingly, prematurity is significantly associated with deviations from normal right-handedness and other motoric laterality (Eames, 1957; Ross, Lipper, & Auld, 1987; Searleman, Porac, & Coren, 1989), and is linked to reduced postural asymmetries at birth in infants born prior to 36 weeks (Gardner, Lewkowicz, & Turkewitz, 1977). In addition, vestibular hypoactivity is frequently noted in premature infants (Ornitz, 1983a, 1983b) who may be deprived of the extensive intrauterine acceleratory environment required for the normal development of the central vestibular system. Prematurity has been linked to delayed motor development, various learning disabilities, and weak motor lateralization. Autism is a complex neurodevelopmental disorder involving a combination of social, communicative, sensory, and motor disturbances. The motor disturbances in particular point to likelihood of dysfunction in the labyrinth or its projection areas (Damasio & Maurer, 1978; Ornitz, 1970, 1983a, 1983b; Ottenbacher, 1982; Vilensky, Damasio, & Maurer, 1981).

Given the likelihood and devastating effects of falls in the elderly, vestibular aging has become an important area of study. Inner ear function, including both hearing and balance, declines with age as the hair cells gradually die off with age. It is estimated that half of inner ear function, and in particular vestibular ganglion cells, are lost by the age of 80. Recent interest has focused on the vestibular loss as a contributor to Alzheimer's disease (Previc, 2013), particularly due to deafferentation of cholinergic inputs emanating from the semicircular canals to higher vestibular and integrative centers.

Taken together, these studies provide preliminary evidence that vestibular stimulation in the form of gravity during early development may be a neurobehavioral determinant of form and function within the mammalian vestibular system by coupling coordinated maturation of angular and linear acceleration systems. It would be interesting to test the hypothesis that restricted or enhanced maternal movements during prenatal life (such as that which occurs during bedrest in humans) lead to neurovestibular alterations or through manipulations mimicking the additional rotation in microgravity. From a developmental programming perspective, it would be valuable to understand more about the lifelong consequences of early vestibular experience in relation to neurodevelopmental disorders, Alzheimer's disease, and other vestibular-related disorders in aging.

Proximal Control of Prenatal Movement: The Amniotic Sac

Embryos of all species exhibit movement prior to birth or hatching (Bekoff, 1976; Coghill, 1929; Hamburger, 1963; Humphrey, 1964; Windle, 1944). Prenatal movements have been classified as either *spontaneous*, that is, occurring in the absence of obvious eliciting stimuli, or *reflexogenic*, that is, evoked by sensory stimulation.

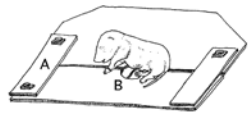
In many species, there is precocial emergence of efferent relative to afferent processes (Coghill, 1929; Hamburger, 1963; Narayanan, Fox, & Hamburger, 1971). Together with classic demonstrations of the independence of fetal motility from sensory input (Carmichael, 1926; Hamburger, 1963), researchers concluded that the prenatal sensory environment played little role in embryonic movement patterns. More recently, however, researchers have challenged this view. There is now ample evidence that the expression of certain early behavior patterns is dependent upon context, and thus on the sensory environment. Dramatic examples of this context dependency are seen when particular environmental manipulations, such as substrate, temperature, or body position, unmask behavior patterns precocially, that is, considerably before or prior to their normal time of emergence (Bradley & Smith, 1988; Fentress, 1981; Hall, 1979; Provine, 1981; Stehouwer & Farel, 1984, Thelen, 1986). Conversely, behaviors that are present in early life but disappear with maturity can be reinstated with specific contextual manipulations (Bekoff & Kauer, 1984); Smotherman & Robinson, 1989; Thelen & Fisher, 1983; Thelen, Fisher, & Ridley-Johnson, 1984). Thus, the proximal environment is an important control parameter for the expression of behaviors during development, even during prenatal life (Thelen, 1988).

The prenatal environment of birds and mammals consists of fluid-filled membranes housed within an egg case or a maternal uterus. Recall that gestational changes in this environment, coupled with rapid prenatal growth, may augment the magnitude of stimulation around the time of birth or hatching. These late gestational changes also create an increasingly constrained prenatal environment that, in turn, necessitates increased energy expenditure during fetal movement (Smotherman & Robinson, 1986). The "restraint" hypothesis receives support from studies of spontaneous prenatal movement in both rats and chicks in which there is general agreement that embryonic behavioral activity within the amniotic sac is attenuated compared to activity following sac removal (Bekoff & Lau, 1980; Narayanan et al., 1971; Oppenheim, 1972; Smotherman & Robinson, 1986). Smotherman and Robinson (1986) observed that synchronous movements of different body parts (also termed "complex movements" by these authors) were more frequent when fetal rats were released from the amniotic membranes into a warm saline bath. Stochastic modeling suggested that complex movements were more than random ensembles of individual body movements; they were presumed to show an emergent organization in a less constrained prenatal microenvironment (Robinson & Smotherman, 1987). In this view, the amniotic sac and uterus constitute an "environmental limitation" that hides or rechannels the expression of an inherent behavioral organization.

Under some circumstances, however, the amniotic sac may *promote* the expression of organized behavior patterns. The facial wiping response to intraoral fluid infusion could be readily elicited on Day 20 of gestation in fetal rats tested either within, or externalized from, the amniotic sac. If the amniotic sac was intact, this species-typical action pattern could be elicited 1 day earlier in gestation (Robinson & Smotherman, 1991). These authors argued that the amniotic sac provides "scaffolding" which reduced head movement and thereby promoted paw-face contact during facial wiping. Thus, the amniotic sac facilitated the expression of prenatal behavior patterns. Clearly, the restraint hypothesis does not fully explain the influence of the amniotic context on fetal behavioral organization.

In a study of the proximal control of overall body movement in prenatal rats, we found that removal of the amniotic sac alters the temporal structure of spontaneous movement in fetal rats. These observations led us to propose that the amniotic sac normally plays a role in the patterning of prenatal movement. Using techniques for in vivo observation of fetal behavior as described earlier, Day 21 rat fetuses were exteriorized from the uterus, with umbilical connections to the dam intact, and videotaped for 15 min either (a) through the intact amniotic membranes or (b) following removal of the membranes (Fig. 2.4). Analysis of fetal behavior categories replicated the findings of previous investigators: Movements of the head, forelimbs, and rear limbs were

a Gestational Day (G) 21 Fetus on Observation Platform

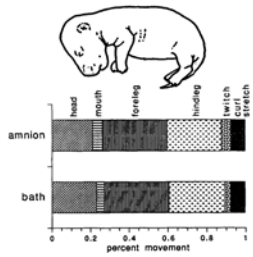


b Frequency of Movement Categories (Bath vs. Amnion)

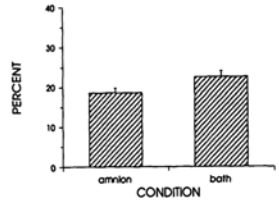
Behavior Category	Group (*Mean* ± *SD*)		*t* test[a]
	Bath	Amnion	
head	91.5 (33.8)	61.0 (23.9)	$t = -2.17$; $p < .05$
foreleg	130.6 (29.8)	98.3 (23.7)	$t = -2.49$; $p < .05$
rearleg	115.1 (36.7)	83.7 (29.7)	$t = -1.95$; $p < .05$
mouth	6.9 (5.9)	10.9 (10.9)	$t = 0.93$; *ns*
twitch	12.0 (7.5)	16.6 (10.8)	$t = 0.99$; *ns*
curl	35.4 (29.1)	25.3 (17.4)	$t = -0.88$; *ns*
stretch	1.6 (1.4)	0.7 (.7)	$t = -1.81$; *ns*
total	393.1 (112.0)	296.4 (92.6)	$t = -1.95$; $p < .05$

Note. $df = 15$.

Movement Types Expressed as a Percentage of Overall Behavior



Complex Movements



c Gestational Day (G) 21 Fetus on Observation Platform

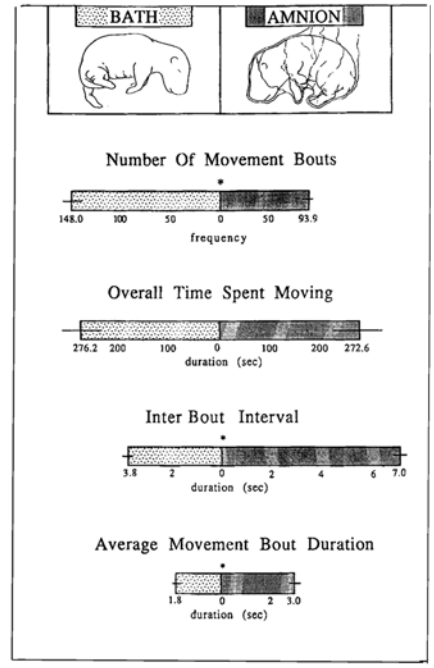


Fig. 2.4 Proximal control of fetal behavior. Rat fetuses were positioned on a platform constructed from two pieces of plastic and hinged at one end (**a**) to enable positioning of the surface between the target fetus and litter. A hole (**b**) in the center of the platform allows passage of the uterine-placental attachment to the dam. (**b**) *Upper image*. Frequency (±sd) of individual behavior categories for fetal rats observed in Bath or Amnion. *Lower left*: Frequency of individual behavior categories for fetal rats observed in Bath or Amnion expressed as a percentage of overall behavior. *Lower right*: Frequency (±se) of complex movements, defined as two or more co-occurring movement types, for fetal rats observed in Bath or Amnion expressed as a percentage of overall behavior. (**c**) Temporal parameters of movement bouts for fetal rats observed in Bath (*lefthand bars*) and Amnion (*righthand bars*) conditions. From *top* to *bottom*: Number of movement bouts, Total time spent moving, Interbout interval, and Bout duration. (Ns = 9 or 10 per group.) (Modified from Ronca et al., 1996)

significantly increased by sac removal, as was the total frequency of behavior categories and the simultaneous occurrence of different behavior categories. Frame-by-frame analysis of videotaped behavior revealed that amniotic sac removal increased the frequency of movement bouts without altering the overall amount of time that fetuses spent moving. Movement bout durations ranged from 50 ms to 70 s. The average duration of movement bouts was significantly reduced for fetuses lacking the amniotic sac as compared to fetuses within the sac, as was the overall distribution of movement bout durations. Frequency distributions of movement bout durations and interbout interval (IBIS) revealed that sac removal significantly increased the occurrence of short (1–2 s) movement bouts and reduced the frequency of protracted movement bouts and interbout intervals (>10-s duration).

Collectively, these findings indicate that quantitative dimensions of fetal rat movements are influenced by proximal features of the uterine environment. During prenatal life, the amniotic sac may normally prolong individual movement sequences by providing proprioceptive feedback to the fetus. Removal of the sac would remove this source of proprioceptive input, and thereby reduce the incidence of protracted movements

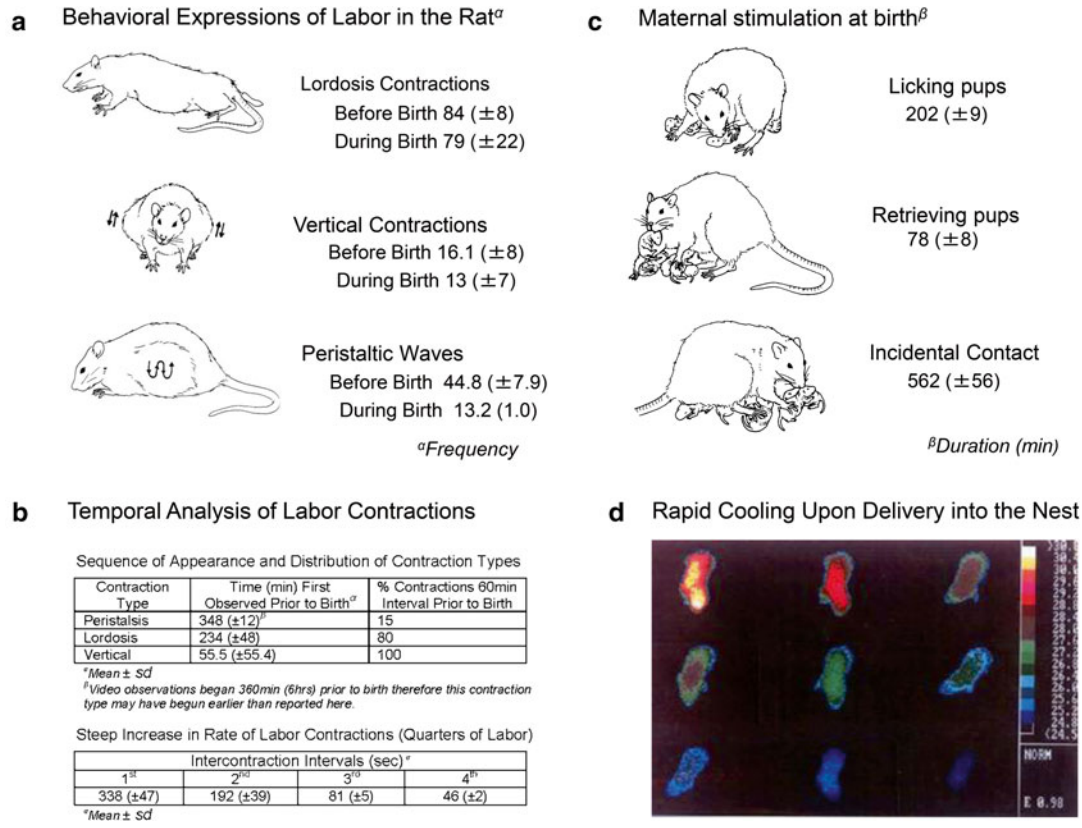


Fig. 2.5 Parturition stimulates fetuses in utero and upon delivery. (**a**) Behavioral expressions of labor in the rat and quantification of contraction types before and during labor (Ronca et al., 1993; Rosenblatt & Lehrman, 1963). (**b**) Intercontraction intervals across quarters of prebirth labor show that as the birth of the first pup approaches, contractions occur at increasingly shorter intervals. (**c**) The mother's peripartum care exposes pups to discrete sensory stimuli. Tactile and vestibular stimulation associated with licking, retrieving, and incidental contact. (**d**) Rapid postpartum cooling upon delivery into the nest. Consecutive thermographic images of a single pup, from *left* to *right*, were taken at 90 s intervals postpartum. The temperature scale on the *right* of the figure indicates that, within 15 min of birth, pup skin temperature plummeted from 37.5 °C (in utero) to 25 °C. Pups remain at room temperatures until the dam completes delivery of the entire litter, averaging ten pups over up to 136 min duration (Ronca et al., 1993). Postdelivery, the dam begins to retrieve pups into a coherent nest and warm them. (Modified from Ronca et al., 1993 and Alberts, Blumberg, & Ronca, 1992)

produced by fetuses. While there are alternative explanations for our findings, this possibility receives strong support from the pattern of results that emerged from our temporal analysis: The average duration of movement bouts in fetuses tested without the amniotic sac was significantly longer than those tested within the sac. In addition, comparison of the entire distributions of movement bouts for each group revealed significantly longer movement bout durations in the Amnion group. Taken together, our results indicate that the total amount of motor output is unaffected by sac removal, but that the *patterning* of movement bouts changes dramatically.

Birth Experiences

The mammalian birth process exposes the fetus and newborn to new forms and levels of sensory experience. During labor (Fig. 2.5) fetuses are pitched and rotated by uterine contractions, and repeatedly exposed to powerful compressions. As the newborn is squeezed through the birth

canal, the pressures are strong enough to change the shape of the newborn's head. Upon delivery from the birth canal the dam removes the birth membranes, enabling air to reach pups' nares for the first time. She licks and handles pups, removing amniotic fluid from the skin as she lifts and rotates them, providing extensive cutaneous stimulation and bouts of linear and angular acceleration. The postpartum thermal environment is much cooler than the intrauterine environment (21 °C vs. 37.5 °C), causing thermally fragile newborn pups to cool to room temperature within moments of being born (Alberts, Blumberg, & Ronca, 1992; Fig. 2.5). Once the entire litter has been delivered, a process that typically lasts more than 1 h, the rat dam gathers her pups into the nest and warms them (Ronca et al., 1993).

The human perinate is similarly exposed to physical stimuli associated with labor contractions and postpartum handling. Sensory development is relatively more advanced in the human baby than in the infant Norway rat (Alberts & Ronca, 1993; Ronca & Alberts, 1995a), providing additional channels for pre- and postnatal sensory input, especially audition and vision. Although our discussion is focused on the physical stimuli that impinge upon the fetal and newborn Norway rat, it is important to note that our intention is to provide a general model of perinatal stimulation that does not preclude a role for stimuli arising from other sensory modalities.

As we reported earlier for intrauterine stimuli, we created 'simulators' of birth stimuli. We observed robust bradycardia and motor responses, controlled bouts of mechanical compression to match contractions of the uterus during labor, stroking to mimic maternal licking at birth, and cooling to match the postpartum thermal environment (Ronca & Alberts, 1994, 1995a).

Effects of Birth Stimuli on Postpartum Adaptation

Upon leaving the womb, a challenge common to mammalian offspring is the initiation of novel behaviors that are vital for survival (Lagercrantz & Slotkin, 1986; Mellor & Lentle, 2014; Ronca, Abel, & Alberts, 1996). The first breath of air, the first nipple attachment, and the expression of learning are all essential beginnings to the fetus's adaptation to postpartum life. Yet, how these behaviors emerge and quickly set into motion remain mysteries. We believe that a psychobiological perspective on postpartum adaptation offers important insight into these fundamental puzzles.

Breathing (*pulmonary respiration*). Figure 2.6 illustrates our approach to understanding the role(s) of birth stimuli on postpartum function. Fetuses were externalized from the uterus, as described previously, and the amniotic sac was gently removed. The fetus was placed in a small cup, and while maintaining intact umbilical connections to the dam, raised above the heated waterbath to provide access to air. Fetuses were exposed to one of three elements of birth: (1) Compressions simulating uterine contractions; (2) Cooling to nest temperature at birth; or (3) Umbilical cord occlusion. Fetuses in a control condition were exposed to air heated to intrauterine temperature (37.5 °C). Figure 2.6 shows that fetuses in each group displayed some respiratory behavior, but only Compression significantly elevated respiratory rate compared to the other experimental conditions. In a second study, pups were exposed to occlusion combined with the other birth factors. Pups in each group were respiring after 1 h, except for pups receiving umbilical cord occlusion without additional stimulation. The 100 % mortality rate of the Cord Occlusion-alone group was reversed by combining cord occlusion with compression (with or without cooling), but not with combined cord occlusion and cooling. Pups exposed to all three factors (compression, cooling, and cord occlusion) breathed at rates identical to those of normal, vaginally delivered pups (Ronca & Alberts, 1994, 1995b). In summary, the absence of prenatal mechanical stimulation greatly compromised the viability of postnatal rat pups. Indeed, fetuses that were exposed to cord occlusion alone never displayed reliable breathing, and did not survive the first postpartum hour. The general significance of this finding is buttressed by previous

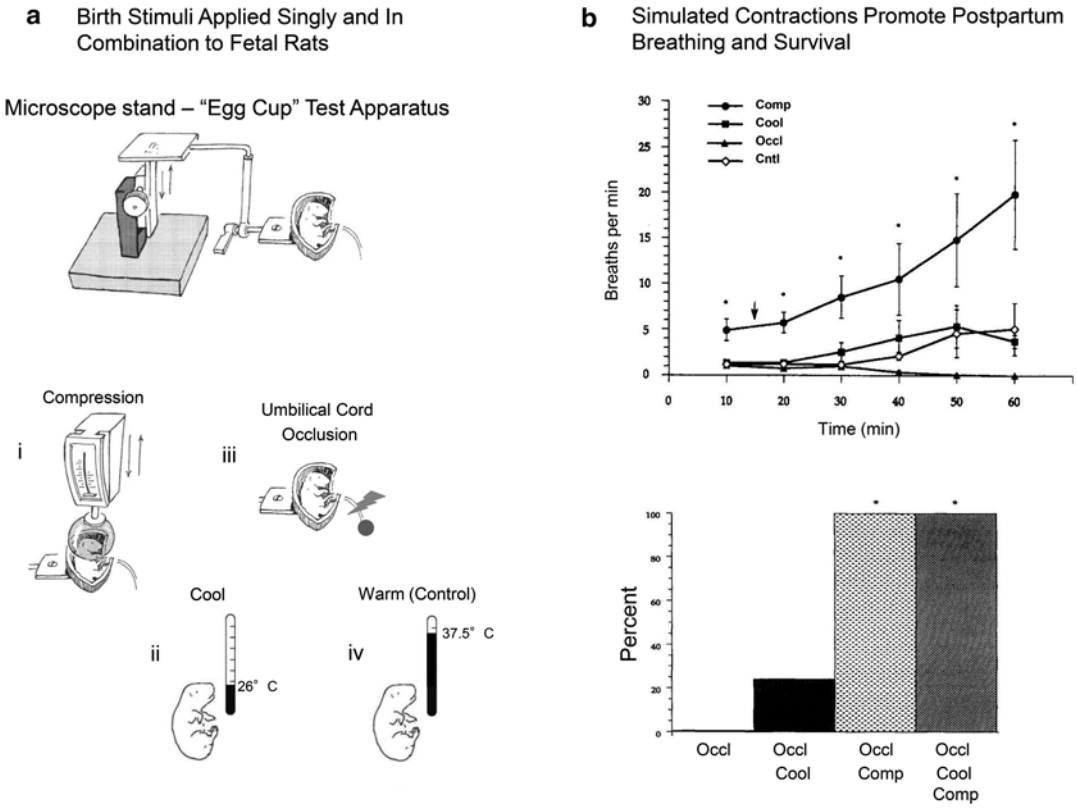


Fig. 2.6 Effects of birth stimuli on pulmonary respiration in newborn rats. (**a**) Methods. *Left panel* (*upper image*). Late gestation (G21) rat fetuses were externalized from the uterus with intact umbilical connections to the dams and tested in an "egg cup" attached to microscope stand, permitting fine vertical control over the position of the fetus positioned with access to air above the mother and heated waterbath. This configuration prevented tension from being applied to the umbilical cord and stabilized the fetus during controlled, reliable application of simulated birth stimuli and monitoring of respiratory movements. *Lower images*. Birth stimuli were: (*i*) simulated uterine contractions produced with a small scale calibrated to deliver 15 mmHg compressions (20 s/min for 15 min), (*ii*) cooling (26 °C), (*iii*) umbilical cord occlusion applied with a microvascular clamp, and (*iv*) a control condition in the surrounding air was heated to intrauterine temperature (37.5 °C). (Modified from Ronca & Alberts, 1994.) (**b**) Breathing and Survival. *Right panel* (*upper graph*). Breaths per minute (mean ÷ se) plotted across ten consecutive 10 min fetal rats in the Compression ($n=10$), Cooling ($n=9$), Control ($n=8$), or Umbilical Cord Occlusion ($n=8$) conditions. All subjects were externalized into heated air (37.5 + 0.5 °C), except for those in the Cooling Condition (26 + 0.5 °C). The *arrow* indicates termination of the compression stimulus. *Right panel* (*lower graph*). Percentage of subjects respiring after 1 h following exposure to umbilical cord occlusion alone (*leftmost bar*), or to umbilical cord occlusion combined with cooling (*second bar*), compression (*third bar*), or cooling and compression (*rightmost bar*). (Modified from Ronca & Alberts, 1995a, 1995b, 1995c)

studies of sheep, species born more precociously than either rats or humans, in which cord occlusion was identified as the major trigger for postpartum respiration. Our findings clearly show that compressions simulating labor contractions are required for maintaining postpartum breathing in rats.

Building a Birth

The results of this study, in which we looked at birth stimuli singly or in combination, identified a role for labor contractions in the emergence of postnatal breathing in the rat. We next expanded our methods to incorporate maternal licking to

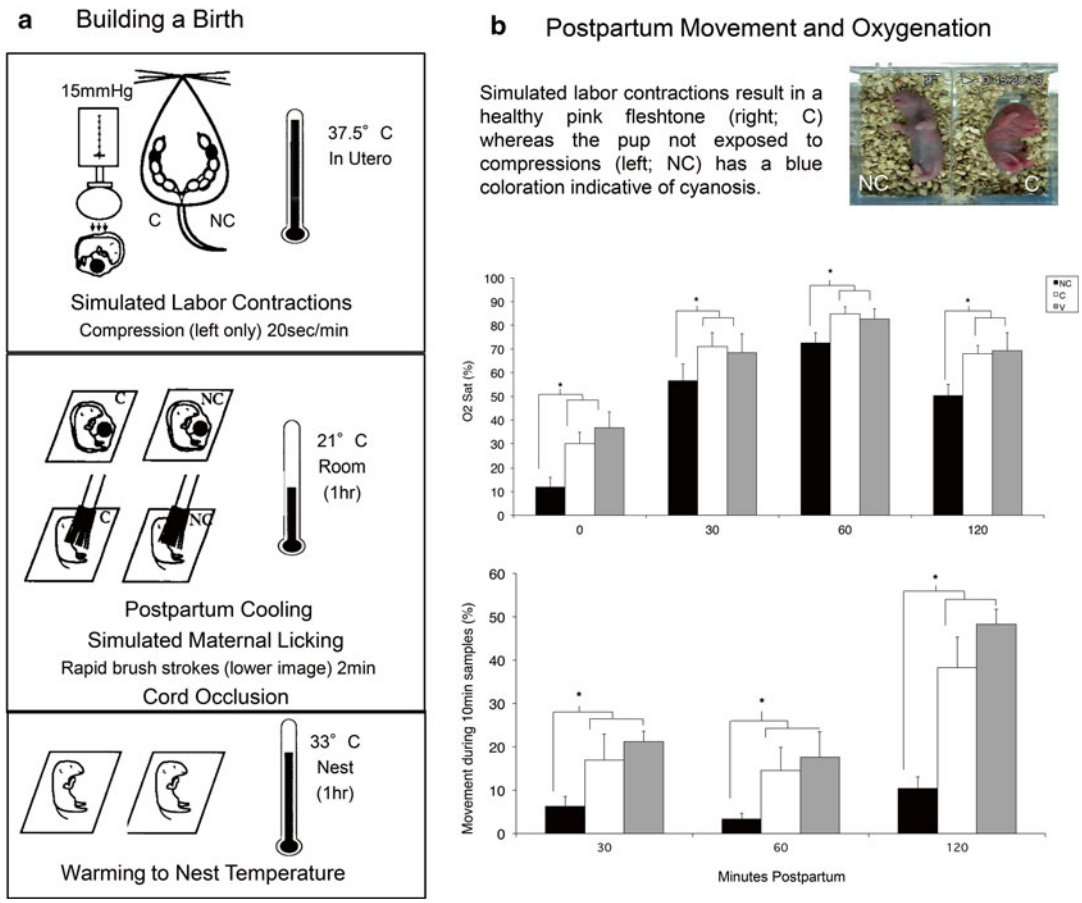


Fig. 2.7 Building a birth. *Upper panel*. Simulated labor contractions (Compressed condition; C) are applied to fetuses in one of the dam's paired uterine horns (*left*) but not to fetuses in the opposite (Noncompressed condition; NC) horn (*right*). *Middle panel*. A surgical Cesarean delivery is performed, pups are placed on a gauze pad at room temperature, administered 2 min of stroking with a brush to mimic licking by the dam, and their umbilical cord is occluded with a microvascular clamp. (**b**) Postpartum movement and oxygenation. *Upper image*. Differential skin coloration of compressed and noncompressed pups at 1 h postdelivery. *Middle image*. Oxygen saturation is comparable in vaginally delivered and compressed pups, but significantly reduced in noncompressed pups. Compressed and vaginally delivered pups exhibit two- to four-fold more activity than Noncompressed controls that persists throughout 2 h postpartum

our suite of birth stimuli, and the process of warming to nest temperature typically initiated by the dam about 1 h after birth, when she has completed delivery of the litter. Figure 2.7 illustrates our approach and findings. We conducted several comparisons of Compressed (C) and Noncompressed (NC) delivered offspring exposed to each of the birth stimuli, varying only compressions. Cutaneous stimulation associated with maternal licking was found to be effective in stimulating postpartum breathing, but did not replace the effects of compression. First, skin coloration is vastly different in C and NC pups, shown here at 1 h postpartum. The blue (cyanotic) skin tone of the NC pups is associated with depressed oxygen saturation, as measured with a clinical blood gas analyzer, that persists through the last measurement at 120 min postpartum. Postpartum movement was similarly depressed in NC pups as compared to C and V pups, further confirming the fundamental importance of mechanical stimulation associated with labor.

Suckling at Birth

After establishing breathing, the newborn's next challenge is to obtain nutrients and water via suckling. We applied our paradigm for analyzing the role of birth stimuli in postpartum adaptation to examine the effects of prenatal compressions and ambient temperature on the newborn rat's first apprehension of a mother's nipple, the first act of suckling (Abel, Ronca, & Alberts, 1998). Fetuses were externalized from the mother's body and exposed to the sequence described in Fig. 2.8. In this study, however, we exposed pups to one of three biologically relevant temperatures: Newborns were exposed to either: (1) Room temperature (21 °C), (2) Nest temperature (33 °C), or (3) Intrauterine (37.5 °C) temperature (Fig. 2.8). After 1 h postpartum exposure to one of the three experimental temperature manipulations, pups from all groups were placed at nest temperature, and then tested for nipple attachment 90 min and again at 120 min postpartum. The Room Temperature condition contained the sequence of thermal exposures experienced by a vaginally born rat pup under typical conditions. This treatment regime (Room Temperature-Compressed or 'simulated birth'), then, was designed to represent the sequence and duration of stimulation that normally occurs prior to and immediately after vaginal birth, leading to the onset of suckling.

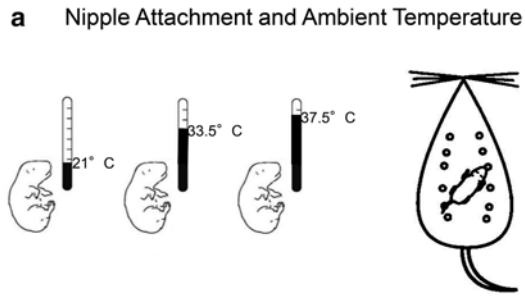
We found that pups exposed to simulated birth and those that underwent normal vaginal delivery showed similar, high rates of nipple attachment (89 and 90 %, respectively; Fig. 2.8). When newborns were tested at 120 min postpartum, simulated contractions increased the probability of nipple attachment in pups exposed to 21 °C relative to noncompressed littermates maintained at the same temperature. Atypically warm postpartum conditions (nest-like or intrauterine) obviated the effects of compression by increasing suckling above the levels seen in noncompressed newborns exposed to the cool condition. Thus, compressions facilitate the achievement of suckling under thermal conditions resembling those typically encountered by the newborn rat.

Simulated Birth Experience Is Sufficient to Induce Odor-Guided Nipple Attachment

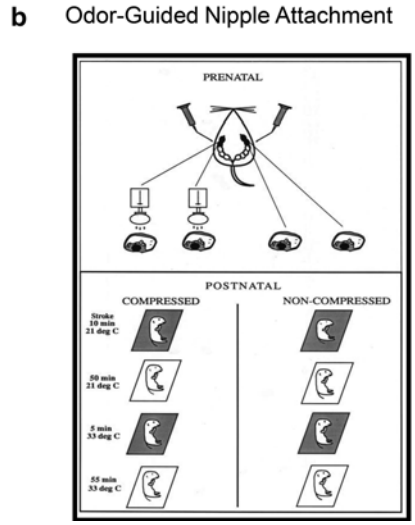
It is known that olfactory cues present on the rat mother's nipples and ventrum are necessary and sufficient to stimulate newborn pups to locate and orally apprehend a nipple and suck. These odor cues can be removed by washing the nipples and surrounding body surfaces that eliminates suckling (Hofer, Shair, & Singh, 1976; Teicher & Blass, 1976). Nipple attachment and suckling by newborns can then be reinstated, by applying to the dam's nipples a distillate of the wash taken from her body, or amniotic fluid, or maternal saliva (Teicher & Blass, 1976). However, other natural and atypical substances were ineffective in reinstating nipple attachment.

Knowledge that amniotic fluid is a sufficient stimulus to elicit the newborn's first nipple attachment led to preliminary considerations of two, mutually exclusive possibilities. One was that the key olfactory stimulus is somehow predetermined and that the newborn is correspondingly and inherently prepared ("hard-wired", so to speak) to detect and respond to the cue. The second possibility was that the perinate responds with nipple attachment and sucking to the amniotic odor stimulus due to previous experience with amniotic fluid. In 1980, Pedersen and Blass, translated these contrasting explanations into an experiment with newborn rats. They reasoned that if amniotic fluid is a behaviorally potent stimulus because the fetus experienced it previously, then another odor, similarly experienced, should have the same behavioral potency as amniotic fluid. They tested this hypothesis by adding a novel, lemon-like substance (citral) to the amniotic fluid, and then testing whether this chemical would rescue the newborn's ability to make its first attachment to the washed nipples of a mother rat. The new odor elicited the sucking behavior and, remarkably, "natural" odors of an unwashed dam were not effective for the citral-treated perinates. The new odor had replaced the natural stimulus!

Pedersen and Blass' (1982) study provided important new insights into the initial plasticity



Group	% attached
Vaginal birth (22°C)	90
Cesarean birth	
Room (22°C)	
Compressed	89*
Noncompressed	44
Nest (33°C)	
Compressed	56
Noncompressed	67
Intrauterine (36°C)	
Compressed	89
Noncompressed	78



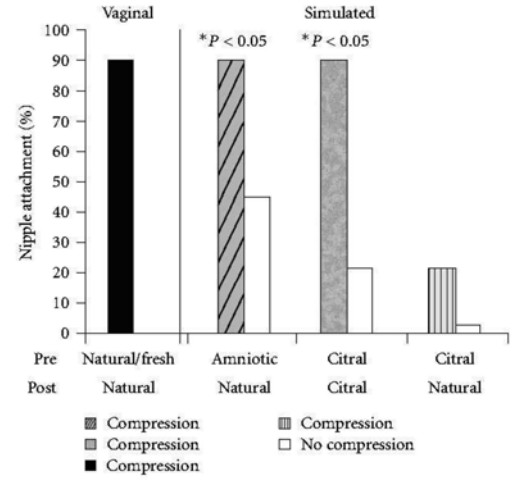


Fig. 2.8 (**a**) Simulated birth promotes the onset of suckling. *Upper leftmost image*. Perinatal rats underwent the simulated birth process shown in Fig. 2.7 and their nipple attachment abilities were tested at 2 h postpartum, except that we manipulated postpartum ambient temperature using one of three biologically relevant temperatures. Newborns were exposed to the cool room temperature environment (22 °C) or to a warmer temperature maintained at nest (33 °C) or intrauterine (36 °C) temperature. After 1 h postpartum exposure to one of the three temperature regimens, pups from all groups were placed at nest temperature then tested for nipple attachment. The 22 °C condition contained the sequence of thermal exposures experienced by a vaginally born rat pup under typical thermal conditions. The treatment regime, then, was designed to represent the sequence and duration of stimulation that normally occurs prior to and immediately after vaginal birth, leading to the onset of suckling. *Upper rightmost image*. Percentage of compressed and noncompressed newborn pups at each ambient temperature attaching to nipples of anesthetized dams (*$P<0.05$). Suckling was dramatically enhanced in compressed pups that underwent the naturalistic cooling episode. (**b**) Odor-guided nipple attachment. Sequence of perinatal manipulations prior to tests of respiration and nipple attachment. *Left image*. The *top portion* of the figure depicts injections of citral or saline into individual amniotic sacs, which was followed by compressions or no compressions (control). Pups were then delivered by Cesarean section. Over the next 2 h, individual pups were treated with thermal and odor regimes designed to mimic early postnatal events, shown in the *lower sections* of the figure. For some pups, citral was present prenatally and postnatally. Saline was used on the gauze pads of the control pups (see text). The *grey* and *white* areas around each pup depict the olfactory treatment (citral or saline control) of the moistened pads. *Lower right image*. Percentage of pups attaching to a nipple of an anesthetized dam during a standardized suckling test. Shown below the *horizontal axis* are the prenatal olfactory conditions for each group and the *lower row* shows the odor conditions during postnatal treatments. The *leftmost* (*black-filled*) *bar* illustrates the baseline rate of attachment by vaginally delivered pups in these nipple attachment tests when the pups were exposed prenatally to natural amniotic odors and tested with a natural-scent dam (Vaginal group). The groups that received simulated birth experiences with different odors were also differentiated by the presence or exclusion of compressions. Within the set of Simulated birth groups, pups compressed in the presence of natural amniotic odors attached to natural-scented dams at rates comparable to the vaginally delivered pups, but those that lacked compressions did not. Pups receiving compressions in the presence of citral (*grey bar*) also attached at the high rate to a citral-scented dam, but uncompressed pups in that group did not. The *rightmost*, *striped histogram* shows that pups receiving compressions in the presence of citral did not attach to natural-scented dams

of the newborn rats' sucking, especially the specification of the cues that can activate and direct the behavior. Their findings created a host of new questions. It seems clear that the establishment of the olfactory control of sucking is determined by the experiences of the perinatal rat pup, but the essential experiences for establishing the newborn's sucking responses to maternal cues have not been clear.

We used our simulated birth paradigm to demonstrate that specific components of maternal stimulation are sufficient conditions for the odor learning that established the newborn's suckling responses to maternal cues (Alberts & Ronca, 2012). We reasoned that if the outcome of the simulated birth experience is equivalent to a natural delivery, then a perinatal sequence of experiences in association with an otherwise neutral olfactory cue should lead to rates of nipple attachment to that cue, similar to those of vaginally delivered newborn rat pups to the odor of amniotic fluid.

Figure 2.8 shows the protocol used in this experiment. Warm citral solution was injected into the amniotic fluid of all subject fetuses immediately following externalization of the uterine horns as described earlier. Prenatal exposure conditions are shown on the top. The shaded areas indicate the presence of citral in the amniotic fluid. Fetuses in one horn were compressed while fetuses in the opposite horn were not. Postnatal exposure conditions are shown in the lower panel. The shaded rectangles represent postnatal citral exposure. Compressed and noncompressed pups were exposed to citral for 10 min. accompanied by a brief period of stroking to stimulate breathing. Citral was then removed and, to mimic birth conditions, pups remained at room temperature (21 °C) for an additional 50 min. During the second hour, pups were exposed to nest temperature (33 °C), with citral presented for the first 5 min of the hour. Figure 2.9 shows that exposure to citral virtually eliminated attachment to fresh (normal, nonscented) nipples, regardless of exposure to compression (left). In contrast, the percentage of pups attaching to citral-scented nipples was dramatically increased by compression, relative to noncompressed subjects (right). Importantly, the attachment rate for these pups is equivalent to that of compressed pups without citral exposure, and to those of vaginally delivered pups to normal nipples.

This study (Alberts & Ronca, 2012) demonstrates that the experience of a simulated birth, quantitatively comparable to a natural, vaginal birth, is sufficient to establish a conditioned response to an odor that is expressed as nipple attachment and the onset of sucking in an otherwise naÏve newborn.

Catecholamines and the Transition from Fetus to Newborn

These studies link the major postnatal milestones of pulmonary respiration and suckling to birth experience. We sought to determine the mechanisms underlying the effectiveness of birth stimuli in helping to promote and organize the fetal-to-neonatal transition. Using our simulated birth model, we tested the hypothesis that compressions simulating labor contractions elicit catecholamine release in the newborn rat. We analyzed the plasma catecholamines, epinephrine (E), and norepinephrine (NE) in newborn rats (0–2 h old) following vaginal birth, Cesarean section with simulated labor contractions, or Cesarean section without labor contractions (Ronca, Abel, Ronan, Renner, & Alberts, 2008; Fig. 2.9). Only pups exposed to actual or simulated labor showed an immediate, dramatic release of E and NE. In all groups, the catecholamine surge was short-lived.

Labor contractions do more than move the fetus through the birth canal. Whether by design (natural selection) or by incidental effect, contractions provide a form of stimulation that serves to facilitate two vital neonatal achievements: pulmonary respiration and suckling. Birth stimuli, that is, the range, levels, and patterns of stimulation that comprise the birth process, might have multiple roles in the successful transition from fetal to postnatal life (Lagercrantz & Slotkin, 1986; Ronca et al., 1996; Fig. 2.9). Our simulated birth model incorporates actual forms and levels of sensory and physiological stimuli to which the

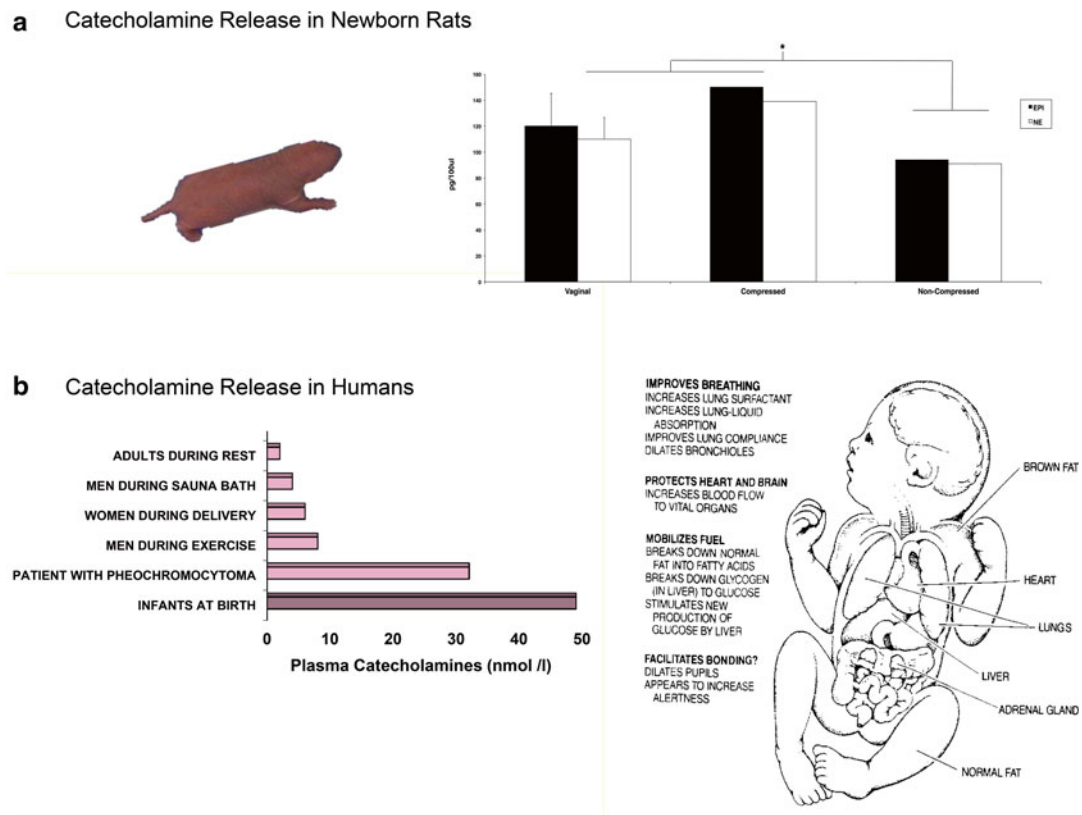


Fig. 2.9 (**a**) Labor-induced catecholamines facilitate the transition from fetus to newborn. (**a**) Catecholamine levels at 0–2 h-old following either: (a) vaginal birth, (b) Cesarean section with simulated labor contractions, or (c) Cesarean section without labor contractions. Pups exposed to actual or simulated labor showed an immediate and profound postpartum rise in norepinephrine and epinephrine, to levels up to 35 % greater than those of noncompressed pups. (Adapted from Ronca, Abel, Ronan, Renner, & Alberts, 2008). (**b**) Catecholamine levels in adults during a wide variety of activities (*first* to *fourthmost bar* from the *top*) or in a patient with a catecholamine-producing tumor (*fifthmost bar*) are dwarfed by the catecholamine surge of infants at birth (*lowest bar*). *Upper right*. The "stress of being born," a catecholamine driven physiological response to labor and squeezing through the birth canal evokes a broad range of positive physiological changes in the infant at birth. (Reprinted with permission from Lagercrantz & Slotkin, 1986)

rat is exposed during natural vaginal birth, enabling us to specifically parcel out the effects of labor on postpartum functions.

The experience of labor is associated with a number of positive neonatal outcomes, including lung compliance, respiratory integrity (Faxelius, Hagnevik, Lagercrantz, Lundell, & Irestedt, 1983; Irestedt, Lagercrantz, & Belfrage, 1984; Van den Berg, Van Elburg, Van Geijn, & Fetter, 2001), blood flow (Faxelius, Bremme, & Lagercrantz, 1982), resistance to oxidative stress (Buhimschi, Buhimschi, Pupkin, & Weiner, 2003), neonatal neurological condition (Otamiri, Berg, Ledin, Leijon, & Lagercrantz, 1991), and complex global EEG patterns (Kim et al., 2003). Human infants are particularly responsive to odors emanating from their mother's nipple/areola region and can identify the nipple by smell (Porter & Winberg, 1999; Varendi, Porter, & Winberg, 1994). Amniotic fluid and breast odors are regulators of infant sucking behavior, comfort, and distress reactions (Doucet, Soussignan, Sagot, & Schaal, 2012). Learning about natural breast odors is enhanced in neonates that experience labor contractions, possibly mediated by NE (Varendi, Porter, & Winberg 1996, 2002).

Together with the results reported herein, these studies support the view that prenatal events associated with labor initiate a cascade of neural, physiological, and behavioral changes that assist the neonate's successful transition to postnatal life events that assist the newborn infant's adaptation to the extrauterine world.

Reflections and Future Directions

In this chapter, we have presented four historical perspectives and recent advances in developmental and clinical research that have influenced our research on intrauterine and birth experience. We also describe our studies of intrauterine and birth experience, shaped by classic works on the role(s) of experience in early development (Gottlieb, 1971a, 1973), and the construct of ontogenetic adaptation (Oppenheim, 1981, 1982). Over the past 20 years, the concepts, approaches, and even language of the perinatal and newborn sciences have changed dramatically. We discuss research on intrauterine determinants of adult health and disease, and the recognition that inputs from the environment can modulate gene activity within and across generations. Collectively, these four scientific traditions afford us new conceptions of how early naturally occurring experiences form pathways to (rather than immutable programs for) later life outcomes given the inherent complexity in identifying and understanding the intervening mechanisms.

These kinds of considerations are particularly helpful for providing a common biological framework within which to examine human development. They also help make animal models more accessible to the human condition, because it is easier to specify the bases of similarities and differences. As students of mammalian development, we hold an inclusive view of the basic processes comprising reproduction and development, recognizing commonalities across mammalian species. Indeed, our past initial analyses of rat parturition (Ronca et al., 1993) were shaped by Lagercrantz and Slotkin's (1986) perspective on the "stress" of being born. The results of our experiments with rats resemble their observations with human birth, and other clinical findings on the importance of vaginal delivery for adaptation to the extrauterine world.

When considering the experimental findings from rats in the context of human births and the onset of suckling, we can speculate on the effects of certain obstetric complications. For example, prematurely born infants enter the postnatal world at a stage of development when their sensorimotor system is not yet prepared for suckling. Dramatically different factors and an unnatural schedule of experiences direct the premature baby to suck. Early postnatal development of the preterm infant may require support by intravenous nutrition and then gastric gavage, where there is an absence of pairing between chemosensory cues and nutritive intake (Schaal, Hummel & Soussignan, 2004). This perspective extends to many aspects of experience that normally contribute to the integration of breathing, suckling, and swallowing. Recognition of such differences is an important step toward an appreciation of the factors that contribute to the full epigenesis of human development, which will inevitably illuminate the origins of certain pathologies, particularly those that involve divergence from typical sequences, timing, or pathways of development.

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Yoke Motor Learning in the Fetal Rat: A Model System for Prenatal Behavioral Development

3

Scott R. Robinson

Abstract

A great deal has been learned about fetal sensory experience and associative learning over the past four decades, but far less is known about how experience may contribute to the prenatal development of the motor system. Indeed, the earliest rudiments of behavior consist of seemingly random spontaneous movements, and experimental demonstrations that spontaneous activity can be generated by isolated elements of the spinal cord have promoted the misconception that experience plays little or no role in early motor development. Building upon an animal model that permits direct assessment of behavior in the rat fetus, my laboratory has developed a motor learning paradigm to study how kinesthetic feedback from motor performance can lead to adaptive changes in motor coordination. Fetal limb movement is manipulated with an interlimb yoke, which creates a physical linkage between two limbs. The yoke results in a gradual increase in conjugate limb movements during a 30-min training session. After yoke training, rat fetuses continue to show enhanced coordination of the trained limbs, which is evident in both the timing and spatial organization of limb movements. Moreover, savings in the rate of acquisition also is evident when fetuses experience yoke training in a second session. These findings argue that fetuses are not automatons but rather are responsive to kinesthetic feedback and can alter the frequency, patterning, and coordination of movement in response to sensory challenges and biomechanical perturbations of the motor system.

Keywords

Rat fetus • Motor learning • Coordination • Kinesthesia • Proprioception

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Animal models have figured prominently in the history of research on fetal behavior. Among the earliest efforts to obtain empirical data on fetal behavior were experimental studies by Graham Brown (1915) and Swenson (1926), who developed methods permitting externalization of living fetuses after surgical preparation of the pregnant mother. Similar invasive methods for gaining direct experimental access to living, active fetuses figured prominently over the next decade, including studies of fetal behavior in cats (*Felis catus*) (Coronios, 1933; Tilney & Kubie, 1931; Windle & Griffin, 1931; Windle, O'Donnell, & Glasshagle, 1933), rabbits (*Oryctolagus cuniculus*) (Pankratz, 1931), rats (*Rattus norvegicus*) (Angulo y Gonzalez, 1932; Windle, Minear, Austin, & Orr, 1935), Guinea pigs (*Cavia porcellus*) (Avery, 1928; Carmichael, 1934), and Sheep (*Ovis aries*) (Barcroft & Barron, 1939; Barcroft, Barron, & Windle, 1936). After a hiatus of nearly 30 years, study of the externalized rat fetus briefly resurfaced in the now classic paper by Narayanan, Fox, and Hamburger (1971), and a decade later with methodological improvements for humane surgical transection of the spinal cord in the pregnant rat (Kirby, 1979; Narayanan, Narayanan, & Browne, 1982; Smotherman, Richards, & Robinson, 1984). Research involving the externalized rodent fetus now represents the primary source of information on the motor, sensory, and learning capacities of fetuses in altricial mammals, such as the rat (for reviews, see Alberts & Ronca, 1993; Brumley & Robinson, 2010; Robinson & Kleven, 2005; Robinson & Méndez-Gallardo, 2010; Ronca & Alberts, 1995; Robinson & Smotherman, 1992a, 1995; Smotherman & Robinson, 1988a, 1998).

In the course of early investigations of the associative learning capacities of the rat fetus, William Smotherman and I noticed that fetal rats, externalized into a warm saline bath after chemical blockade of the maternal spinal cord (Smotherman & Robinson, 1991), typically express a coordinated motor response to certain forms of chemosensory stimulation (Smotherman & Robinson, 1987). For instance, if a small volume (20 μl) of milk (actually bovine light cream) is experimentally infused through a fine cannula into the mouth of the fetal rat, it reliably elicits a stretch response that is very similar to the stretch reflex expressed by suckling rats at the nipple. Subsequent investigations characterized the stretch response and the stimulus parameters that were most effective in promoting its expression (Robinson & Smotherman, 1992b). Although the response resembled the form of the neonatal stretch, it was very different in its timing in relation to infusion of milk. During normal suckling at the nipple, the infant rat attaches to the nipple and engages in rhythmic mouthing activity for several tens of seconds ranging up to several minutes before the mother responds by letting down milk, enabling the pup to extract it from the nipple and swallow it (Drewett, Statham, & Wakerley, 1974; Hall & Rosenblatt, 1977). In contrast, the fetal rat that receives an intraoral infusion of milk performs a few mouthing movements, then systematically alters the topography of its motor activity over the next 2–4 min before the stretch response ultimately occurs (Robinson & Smotherman, 1992b). Why the delay? Why do fetal rats not express the stretch response immediately upon ingestion of milk, like their postnatal counterparts?

After many experiments and exploratory studies, we finally resolved the question by recognizing that the ongoing motor behavior of the fetal rat was crucial to the expression of the stretch. The fetus's initial response to intraoral infusion of milk was to perform a few mouth movements and then become relatively quiet. Over the next several minutes, as the fetus continued to express spontaneous movements of head, forelimbs, hindlimbs, and body trunk, the proportion of overall activity that involved hindlimb movement progressively increased from about 35 % of all movements before infusion to a peak of nearly 75 % 2–4 min later. It was at the peak of hindlimb activity that the stretch response occurred, suggesting that progressive change in the topography of fetal movement contributed to expression of the stretch response (Robinson & Smotherman, 1992b). These changes in motor activity were accompanied by changes in activity of the endogenous opioid system, which also occurred in response to

milk infusion (Smotherman & Robinson, 1992a). Blockade of opioid receptors with either nonspecific antagonists, such as naloxone, or antagonists selective for the Kappa class of opioid receptors was effective in preventing expression of the stretch (Smotherman & Robinson, 1992b).

Was the shift in motor activity involving the predominance of hindlimb movements necessary for the stretch to occur? To test this hypothesis, we manipulated sensory experience after infusion of milk in an effort to facilitate hindlimb activity. Two methods were used. In the first, fetuses received stroking with a soft paintbrush directed at the anogenital region. Anogenital stimulation simulates maternal licking of pups and is effective in promoting a reflexive extension of the hindlimbs, referred to as the limb extension response (LER) (Moore & Chadwick-Dias, 1986). In the second approach, we created a physical linkage between the two hindlimbs by gently tying a short length of suture thread to both ankles. Our reasoning was that tethering the two limbs would result in mechanical coupling of the hindlimbs that would generate sensory feedback with each spontaneous hindlimb movement, thereby stimulating more hindlimb activity. As control conditions, fetuses were exposed to comparable brush stroking directed to the head and neck rather than the anogenital region, or were fitted with the hindlimb tether that was immediately divided with scissors. The results were unambiguous: both anogenital stimulation and tethering of the hindlimbs after infusion of milk reduced the latency between milk infusion and the stretch response, and hindlimb tethering increased the proportion of fetuses that expressed a stretch (Robinson & Smotherman, 1994).

Prenatal Development of Kinesthesia

The findings of this curious little experiment had much wider implications. The fact that fetal rats were responsive to an interlimb yoke that created sensory stimulation contingent on motor activity implied that fetal motor behavior could be influenced by somatosensory feedback, including proprioception. This inference contrasted sharply with the widely held idea in developmental neuroscience that sensory feedback during fetal development contributed little to the development of motor behavior. Our result suggested that fetal motor behavior could be shaped by learning.

Whether sensory feedback may contribute to the early ontogeny of organized motor behavior is the source of a long-standing debate in developmental neuroscience. Motor learning, motor training, practice effects, and skill development are routinely invoked to account for behavior change and plasticity in motor control during childhood, adolescence, and adulthood. But it is far less clear whether experience might contribute to fundamental patterns of motor behavior or the neural systems that govern motor activity before birth. It is widely accepted that spontaneous motor activity in avian and mammalian embryos produces beneficial effects on the development of physical anatomy, such as bones, muscles, connective tissue, and integument (Drachman & Sokoloff, 1966; Moessinger, 1983; Muller, 2003). There also is abundant experimental evidence that sensory development is shaped by stimulation during the prenatal period in both avian embryos (Gottlieb, 1997; Lickliter, 1995) and mammalian fetuses (Fifer & Moon, 1995; Kisilevsky et al., 2003; Mennella, Jagnow, & Beauchamp, 2001; Ronca & Alberts, 1994; Schaal, 2005). Receptors and central neural systems needed for gustation and olfaction become functional before birth, even in altricial species such as the rat, and fetuses also are responsive to sounds in utero in humans and precocial mammals. But far less is known about prenatal responsiveness to proprioceptive and kinesthetic information. Can sensory feedback arising from the fetus's own movements alter the coordination and development of fetal motor behavior?

Some of the early pioneers of behavioral embryology argued that even the simplest motor acts are shaped by the prenatal sensory environment and consequences of motor performance (Kuo, 1967). However, experiments in which amphibian embryos were exposed to drugs that abolished muscle activity suggested that there were no long-term behavioral effects after embry-

onic immobilization, which implied that sensory feedback from motor activity was not necessary for normal motor development (Carmichael, 1926; Haverkamp, 1986; Haverkamp & Oppenheim, 1986; Matthews & Detwiler, 1926). This conclusion accorded well with the experimental findings that emerged from Viktor Hamburger's laboratory in the 1960s. These studies demonstrated that movements by the chick embryo were generated by the central nervous system, specifically by localized circuits in the spinal cord, and occurred even when spinal segments were isolated from the brain (by spinal transection) and from sensory feedback (by deafferentation) (Hamburger, Wenger, & Oppenheim, 1966; Narayanan & Hamburger, 1971; Narayanan & Malloy, 1974; Provine, 1972). The fact that embryos could move without sensory feedback seemed to imply that feedback did not contribute to prenatal development of motor systems (Oppenheim, 1972).

For fetuses to be influenced by feedback from motor activity, they first must possess a functional kinesthetic sense. Kinesthesia actually entails many sensory modalities, including responsiveness to somatosensory (touch), vestibular, and proprioceptive stimuli. In contrast to the handful of attempts to deprive non-mammalian embryos of movement, a growing number of studies have demonstrated prenatal function in each of these kinesthetic sensory systems. Responsiveness to somatosensory stimulation, including punctate tactile stimuli and brush strokes to various parts of the body, have been known to evoke fetal responses since the earliest empirical studies (Angulo y Gonzalez, 1932; Carmichael, 1934). The effects of prenatal stimulation of the vestibular system also have been documented in fetal rats gestated under conditions of low gravity during flights of the space shuttle (Jamon, 2014; Ronca & Alberts, 2000; Ronca, Fritzsch, Bruce, & Alberts, 2008; Walton, Harding, Anschel, Harris, & Llinás, 2005; Walton, Heffernan, Sulica, & Benavides, 2007). Critical elements of the third kinesthetic modality—proprioception—also develop in advance of birth, including mechanoreceptors and muscle spindles (Fitzgerald, 1987; Kucera, Walro, & Reichler, 1989; Milburn, 1973). Reduction of buoyancy within the egg can experimentally alter the form and quantity of motor activity in chick embryos (Bradley, 1997). This effect is likely mediated by proprioceptors, as similar effects result from restraint of movement at a single leg joint (Bradley & Sebelski, 2000), or selective elimination of large proprioceptive neurons via pyridoxine toxicity (Sharp & Bekoff, 2015). Fetal rats show similar changes in overall motor activity when released from the restraint of the amniotic sac into an unrestrained fluid medium (Kleven, Lane, & Robinson, 2004; Ronca, Kamm, Thelen, & Alberts, 1994; Smotherman & Robinson, 1986). Experimental findings such as these are suggestive that proprioceptive stimuli can influence fetal motor behavior. In turn, functional proprioceptive senses raise the possibility that motor learning may contribute to the development of organized motor behavior before birth.

Yoke Training as a Model of Motor Learning

To experimentally investigate this possibility, my laboratory modified the hindlimb tethering method used to study the stretch response as an explicit model of motor learning in the fetus. In its modified form, a length of suture was attached to both ankles of a subject fetus, leaving a specific gap between the feet. In E20 rat fetuses (the 20th day of gestation, 2 days before birth, with E0 designated as the day of conception), the average distance between the hindfeet when the fetus is at rest is 8 mm, so the length of the thread between the ankles was set at 8 mm for fetuses tested at this age. This gap in the connecting thread allowed fetuses to move both hindlegs independently, but movement of an individual limb would encounter resistance if the other limb remained motionless or moved in a different direction. We expected that after a period of exposure to the interlimb yoke, fetuses would alter either the amount or patterning of their motor activity to avoid the impediment to independent limb movement.

Fetuses were exposed to interlimb yoke training, or control conditions, during a 60-min experimental session comprising 30 min of yoke training (designated the Training Period)

followed by 30 min after removal of the yoke (the Test Period). (In practice, the yoke was divided by cutting with scissors, providing almost instantaneous removal of contingent limb restraint without handling of the subject.) The results of the first yoke-training experiment are shown in Fig. 3.1. Overall hindlimb activity was elevated in the Yoked group during the last 10 min of the Training Period and the first 10 min of the Test Period (Fig. 3.1, top). Forelimb activity, however, was not affected by the presence or removal of the hindlimb yoke. The increase in hindlimb activity might have indicated struggling on the part of the fetus in response to limb restraint. But we also noticed that patterning of hindlimb activity also changed during training.

To quantify changes in interlimb coordination, we noted the occurrence of each instance of conjugate limb movement (CLM). A conjugate movement occurred when both hindlimbs initiated movement at the same time and moved in the same direction, such that the two limbs appeared to move as if one. Hindlimb CLM also changed during yoke training in dramatic fashion: yoked subjects expressed more CLM than either Unyoked controls (fetuses that were fitted with the interlimb yoke that was immediately divided) or subjects that received No Treatment. While CLM remained infrequent in Unyoked and NT subjects throughout the Training and Test Periods, CLM increased nearly sixfold during Training in Yoked subjects. Moreover, the effect of the interlimb yoke on hindlimb coordination persisted after the yoke was cut, resulting in elevated levels of hindlimb CLM for the next 20–25 min. This effect of yoke training was evident both when examining changes in the absolute frequency of CLM (Fig. 3.1, center) and when CLM was expressed as a percentage of overall hindlimb activity (Fig. 3.1, bottom). In a final analysis, CLM was subtracted from overall hindlimb activity and the remainder compared across the three groups. This comparison indicated no significant changes in non-CLM hindlimb movements during the experimental session in any of the three conditions. Therefore, yoke training appeared to be confined specifically to conjugate movements of the hindlimbs, with no effect on forelimb or non-CLM hindlimb behavior.

The findings from this initial yoke-training experiment confirmed our general expectations. Exposure to the interlimb yoke altered hindlimb activity and appeared to increase the synchronized coordination of movement of the two limbs. It also documented key features of interlimb yoke training that we have replicated many times since (Robinson, 2005; Robinson & Kleven, 2005; Robinson, Kleven, & Brumley, 2008). First, conjugate movements of forelimbs or hindlimbs occur infrequently during spontaneous fetal activity, and continue to be expressed at very low rates in unmanipulated or unyoked subjects. Second, the increase in CLM that occurs as a result of yoke training occurs gradually, not abruptly. One might expect a reflexive struggling response to be expressed immediately after attachment of the yoke to both ankles. Instead, CLM emerges from a background of spontaneous activity as the fetus is exposed to training over a period of time. Third, changes in CLM are restricted to the limbs that experience the contingent constraint of movement. We found no effect of hindlimb yoke training on forelimb behavior. Finally, CLM continues to be expressed after the movement constraint created by the yoke is eliminated. The decline in CLM after the yoke is divided more closely resembles the extinction phase in an associative learning experiment than the sudden cessation of response after termination of a stimulus.

The results of this experiment also raised questions about the specificity of the response, what aspects of limb movement were altered, and whether our categorical coding of CLM could be confirmed by objective characterization of changes in interlimb coordination. To address these questions, we replicated the experiment and subjected video recordings to frame-by-frame motion analysis.

Characteristics of the Learned Motor Response

The motion analysis study employed a similar yoke-training procedure to assess the capacity of the fetal rat to express motor learning. Subjects were individual rat fetuses from five pregnancies that were prepared for direct, in vivo observation

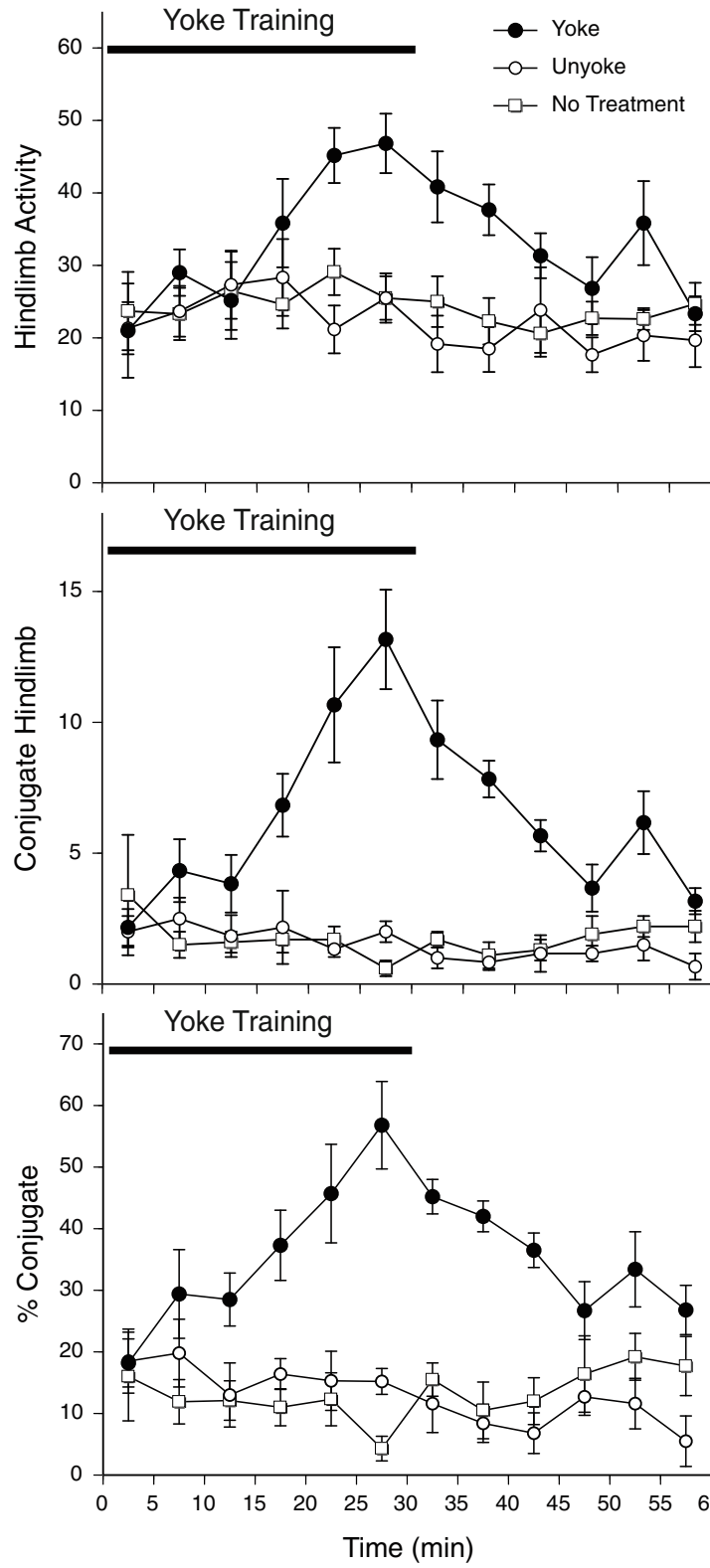

Fig. 3.1 Changes in hindlimb activity (*top*) and conjugate hindlimb movements expressed as frequency counts (*center*) and as a percentage of hindlimb activity (*bottom*) during and after interlimb yoke training on E20. Fetuses received interlimb yoke training (Yoked), were fitted with a yoke that was immediately cut (Unyoked) or remained unmanipulated (No Treatment) during the first 30 min of the session, as indicated by the *horizontal bar*. The yoke was divided at the beginning of the second half of the session in Yoked subjects. *Points* show the means; *vertical bars* depict SEM

on E20. To facilitate video recording of fetal movements, each subject fetus was placed on a horizontal support and held in a supine position with a soft elastic strap that passed over the upper thorax (Fig. 3.2, left). Fetal activity was videotaped at 60 fields/s simultaneously from two camera views during a 40-min experimental session. Fetuses were allowed to move without obstruction for the initial 5 min of the session (Pre-Yoke). The interlimb yoke (length = 8.0 mm) was attached to both hindlimbs at 5 min, at the beginning of the 30-min Training Period. The yoke was divided with scissors at 35 min and fetal behavior recorded during an additional 5 min without constraint of hindlimb movement (Post-Yoke).

Video recordings were analyzed in two phases. Recordings initially were observed during playback (60 fields/s) and scored by visual observation with event-coding software as in the initial experiment, distinguishing individual movements of left and right hindlimbs and CLM. Visual scoring of CLM suggested that fetuses adjusted interlimb coordination between the hindlimbs as we previously found (Robinson, 2005). To quantify changes in interlimb coordination, motion analysis was conducted to compare fetal hindlimb movements before and after yoke training. For both the Pre-Yoke and Post-Yoke periods in each of the five subjects, a 1-min segment comprising the median level of CLM in that 5-min period, as determined by visual coding, was selected for frame-by-frame analysis, yielding a total of 36,000 digitized video fields. Digitizing of video fields and calculation of 3D coordinates and kinematic variables were performed with a dedicated motion analysis system (Peak Performance Technologies, Englewood, CO). This system is accurate in computing 3D paths and velocities of movement (Scholz & Millford, 1993), and has been used in previous kinematic studies of motor activity in avian embryos (Bradley & Bekoff, 1990; Chambers, Bradley, & Orosz, 1995; Watson & Bekoff, 1990). Based on measurement errors of the calibration frame used in this study, accuracy of 3D point coordinates (95 % confidence intervals) was estimated to be 0.011 mm (*X* axis), 0.045 mm (*Y* axis), and 0.008 mm (*Z* axis).

The small size and submerged position of fetal subjects, and the tendency for fetal skin to move relative to underlying joints, precluded effective use of markers to permit automated tracking, so four points on the body of the fetus were manually digitized in each video field: the tip of the left and right hindpaws, the anterior edge of the umbilical cord where it connects to the abdomen, and the tip of the sternum. All of these points were visible from both camera views. After calibration of the space visible from both camera views, a direct linear transformation algorithm was used to calculate the 3D coordinates of each digitized point from its pixel coordinates (Abdel-Aziz & Karara, 1971). Raw digitized data were smoothed using a 60 Hz Butterworth filter. A line passing from the sternum point through the umbilical point was used to define the midline of the fetus's body, with the umbilical point serving as the point of origin (0,0,0 for *X*,*Y*,*Z* dimensions). 3-D coordinates then were transformed and rotated to define the *X* dimension as the fetal midline (rostral-caudal axis, positive values rostral from origin), the *Y* dimension as vertical (dorsal-ventral axis, positive values ventral), and the *Z* dimension as lateral (positive values to left). In this way, hindlimb movements were measured relative to the sternum-umbilical axis, not an external reference frame. From these *XYZ* coordinates, the path of each hindpaw could be traced and its resultant velocity (vector sum of *XYZ* velocities) calculated (Fig. 3.2, right).

Overall hindlimb activity, as determined by visual coding, was analyzed across the eight 5-min intervals of the experimental session. This analysis indicated no significant change in hindlimb activity over time. However, the occurrence of CLM did increase significantly during the Training period, both in raw frequency and when expressed as a percentage of hindlimb activity. CLM occurred at a low rate during the Pre-Yoke period (mean = 3.4 ± 0.7 movements, 10.6 % of hindlimb activity). But CLM increased gradually during the first 10 min of yoke training, which continued during the next three intervals and reached a peak in the last 5 min of Training (19.4 ± 5.2 movements, 49.3 % of hindlimb activity). Moreover, the elevated level of CLM

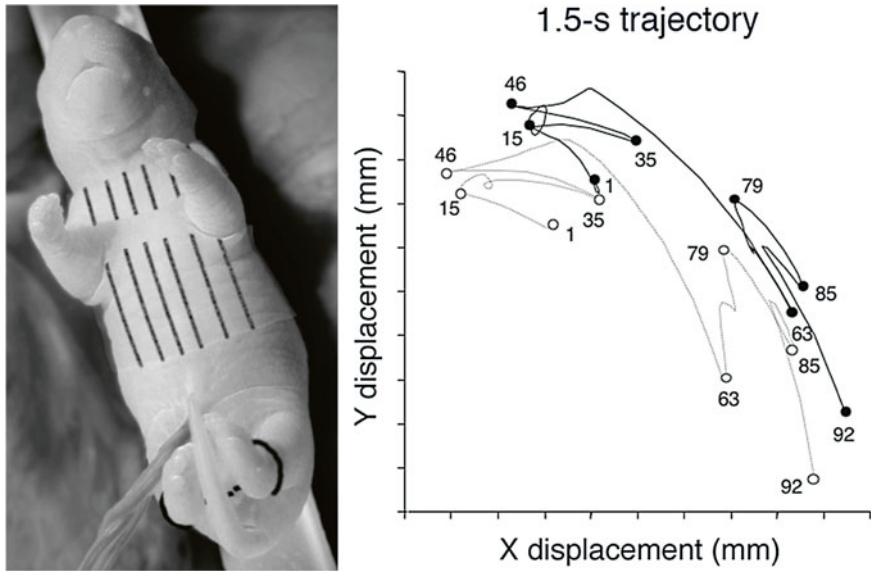

Fig. 3.2 (*Left*) Photograph of E20 rat fetus prepared for interlimb yoke training. The fetus is held in place in a supine position on a submerged platform with a transparent elastic harness marked with *fine lines* to facilitate manual digitizing. Part of the interlimb yoke can be seen as the *dark line* around the fetus's left ankle. (*Right*) Example of hindlimb trajectories in the sagittal (*X*–*Y*) plane during a 1.5 s segment after yoke training. Rostral is toward the *left*, ventral toward the *top* of the graph. The position of right (*solid line*) and left (*dotted gray line*) hindpaws at selected points are indicated by field numbers (1 s = 60 video fields). Note how both paws undergo smooth changes in direction and velocity as they trace parallel paths during conjugate hindlimb activity

persisted after the yoke was cut, during the Post-Yoke period (mean = 15.0 ± 3.3 movements, 43.7 % of hindlimb activity).

Data obtained from visual coding were used to select 1-min segments during the Pre-Yoke and Post-Yoke periods for more detailed motion analysis. During the Pre-Yoke period, 1-min intervals ranged from 0 to 3 CLM; during Post-Yoke, they ranged from 1 to 9 CLM. For each subject, the segments selected for motion analysis represented the 1-min interval with the median number of CLM for the period. To provide a measure of dynamic changes in hindlimb movement before and after yoke training, various kinematic measures were derived from 3D coordinates of the left and right hindlimbs within a 1-s moving window ($N = 60$ video fields per window). The window was advanced in increments of one video field (17 ms). Each 60-s segment thus yielded a time series of 3540 samples.

Using the time series of 3D coordinates obtained from motion analysis, the amount of fetal movement during sample segments was measured in three ways. (a) The percentage of 1-s windows in which a hindlimb moved at least 5 mm was calculated. This measure indicated fetal hindlimb activity during 44.9 ± 7.9 % (mean ± SEM) of the Pre-Yoke segment, compared to 54.2 ± 12.0 % of the Post-Yoke segment. A paired *t*-test indicated no significant difference in this measure of hindlimb activity between Pre- and Post-Yoke segments. (b) The magnitude or vigor of hindlimb activity was measured by calculating the total length of the path traced by each hindlimb during each 1-min segment. The sum of left and right path lengths was 1991.6 ± 504.6 mm (mean ± SEM) during the Pre-Yoke segment and 1958.4 ± 281.2 mm in the Post-Yoke segment. A paired *t*-test similarly indicated no significant difference in the amplitude of movement before and after training. (c) A third measure of hindlimb activity was obtained by calculating the number of peaks (>20 mm/s) in the velocity time series for both hindlimbs. This is similar to methods previously reported for identifying the number of movement units in a

continuous kinematic record (Thelen, 1994). This measure indicated 60.2 ± 12.6 peaks (mean ± SEM) during the Pre-Yoke segment compared to 87.6 ± 21.6 peaks during the Post-Yoke segment. This measure also did not differ before and after training. These three measures of hindlimb activity agree broadly with the findings derived from visual coding, that hindlimb movements were expressed at similar levels before and after yoke training.

Visual scoring of CLM indicated that fetuses adjusted the coordination between hindlimbs to compensate for the physical constraint of movement created by the interlimb yoke, consistent with the findings of our initial yoke-training experiment. A Pearson product–moment correlation between the coordinates of the left and right hindlimbs was calculated in the 1-s moving window (Thelen, 1994). For each window, a correlation of +1 indicated that both hindlimbs were moving in a conjugate pattern, moving in phase through extension and flexion of each limb. Conversely, a correlation of −1 indicated an alternated or antiphase pattern of limb motion. To exclude spurious correlations during periods when neither hindlimb was actively moving, the correlation was included only when one or both hindlimbs moved through a distance of at least 5 mm within the 1-s window (Passive movement of the limbs due to breathing of the pregnant rat and consequent oscillation of the water in the saline bath was the principal cause of low amplitude movements that were excluded by this criterion.)

Moving-window correlations were calculated for displacement of the left and right hindlimbs in each of the three dimensions (*XYZ*), and for the resultant velocities of each hindlimb. The distributions of these correlations before and after yoke training then were compared for each subject (using nonparametric Kolmogorov-Smirnov tests). For displacements in both the *X* (rostral-caudal) and *Y* (dorsal-ventral) axes, all five subjects showed a significant Post-Yoke shift toward more positive values. In the *Z* (lateral) axis, four of five subjects showed a significant positive shift, and four subjects showed a positive shift in correlations of resultant velocity. Strong positive correlations in both limb displacement and velocity reflect a change in interlimb coordination toward synchronized parallel trajectories. This change in movement coordination also was evident in the proportion of moving windows that yielded strong positive correlations. The positive tails of correlation distributions were compared in the Pre- and Post-Yoke segments by paired t-tests. After 30 min of yoke training, displacements of left and right hindlimbs became more highly correlated ($r > 0.8$) in the *Y* axis. Strong correlations ($r > 0.8$) in the *X* axis were marginally significant, but moderately strong positive correlations ($r > 0.6$) increased markedly after training. Strong correlations in the *Z* axis narrowly missed statistical significance, and moderate correlations ($r > 0.6$) also were not different before and after training. Resultant velocities of the two hindlimbs became more highly correlated ($r > 0.8$) after training. Figure 3.3 depicts changes in the distributions of moving-window correlations averaged across all fetal subjects.

One additional analysis provided further evidence that fetuses altered their interlimb coordination specifically to accommodate the characteristics of the interlimb yoke. Because the interlimb yoke enforced a fixed separation between the hindlimbs, fetuses may have learned to maintain that separation after the yoke was removed. The distance between the tips of the left and right hindpaws (L-R distance) was calculated in each video field of Pre- and Post-Yoke segments. Variability in the position in the L-R distance was expressed as the Coefficient of Variation. L-R distance before training was 5.64 ± 0.40 mm (mean ± SEM), which increased significantly to 7.71 ± .74 mm after training. But the CoV of the L-R distance decreased significantly from the Pre-Yoke period (31.8 ± 1.7 %) to the Post-Yoke period (19.8 ± 3.2 %). This finding suggests that the separation between the hindlimbs was more tightly constrained—even after removal of the interlimb yoke—despite the absence of changes in overall movement duration, amplitude, or velocity. Moreover, the distance maintained between the limbs after training (7.71 mm) more closely approximated the 8.0 mm gap enforced by the interlimb yoke. Indeed, although fetuses were observed to move

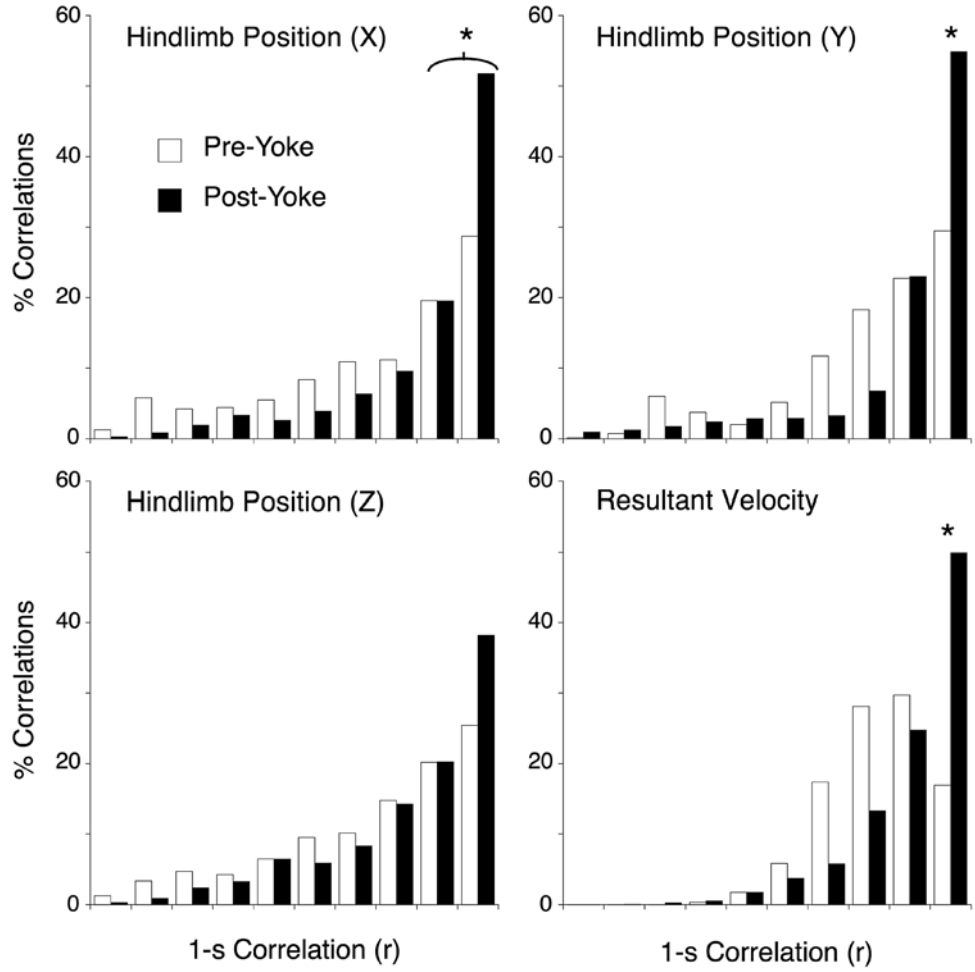

Fig. 3.3 Changes in interlimb coordination before and after yoke training, as measured by correlations between left and right hindpaw position and velocity. Each *panel* presents the *histogram* of Pearson product–moment correlations calculated within a moving 1-s (60 field) window during a 60-s period before (Pre-Yoke) and after (Post-Yoke) interlimb yoke training. The maximum number of correlations in each distribution is 3540, but the graphs exclude windows where neither hindlimb moved more than 5 mm. The X-axis of each histogram shows the full range of correlation values, from -1 to +1. Note the right shift in the distribution of correlations for both position and velocity, indicating more strongly positive correlations resulting from in-phase movement of the two limbs

the hindlimbs with a separation ranging from 1.4 to 22.0 mm, the proportion of time with the two hindlimbs within 6–10 mm of each other (±2.0 mm from the 8.0 mm yoke) increased significantly from 28.8 % before training to 65.5 % after training. Changes in strong positive correlations in hindlimb position and velocity, and measures of the L-R distance, are summarized in Fig. 3.4. Taken as a whole, these analyses of fetal movement suggest that the spatial position and timing of movement of the two hindlimbs became more highly coordinated as a consequence of the interlimb yoke, and that these changes in interlimb coordination persisted after the yoke was cut.

The findings from the motion analysis experiment, and from parallel experiments in our laboratory (Robinson, 2005; Robinson et al., 2008), suggest that a brief period of biomechanical constraint of movement can induce the rat fetus to alter the coordination of spontaneous hindlimb activity. Because synchronous hindlimb

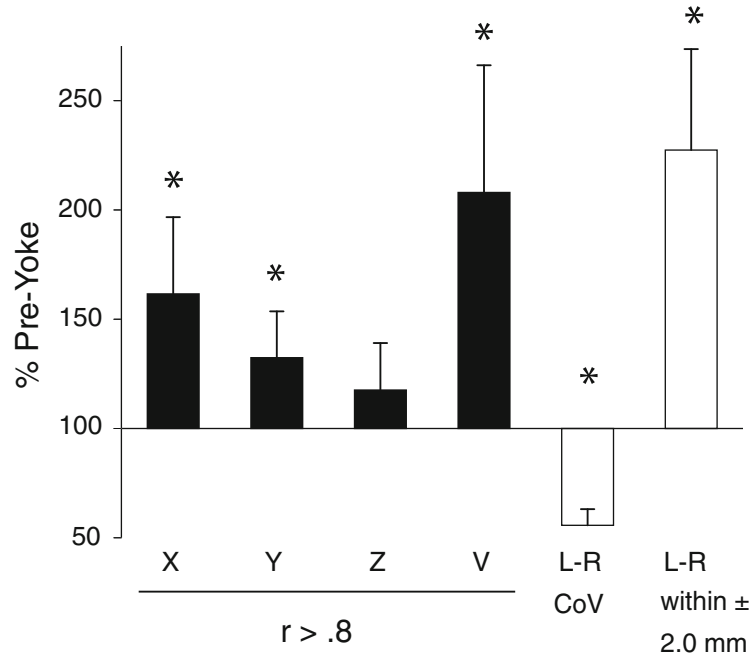

Fig. 3.4 *Black bars* show changes in strong positive correlations in each of three axes of hindlimb position (*X* = rostral-caudal, *Y* = dorsal-ventral, *Z* = medial-lateral) and resultant velocity after interlimb yoke training, measured relative to Pre-Yoke values (100 %). White bars depict changes in the Coefficient of Variation of the distance between left and right hindpaws and the time with left and right hindpaws within 2.0 mm of each other. *Vertical lines* show SEM

movements increase only gradually after application of the interlimb yoke, they cannot be attributed to struggling or other reflexive responses evoked by the presence of the yoke. Moreover, the persistence of synchronous hindlimb movements after the interlimb yoke is removed provides further evidence that the fetus is facultatively modifying the coordination of its limb activity. Fetal hindlimb movements did not become more stereotyped in response to the interlimb yoke. In contrast, they continued to be expressed at the same (or elevated) rates with the same amplitude and the same (or greater) number of velocity peaks. However, variability in the distance between left and right hindpaws decreased after yoke training, and the distance between paws more closely approximated the length of the interlimb yoke itself, suggesting that fetuses coordinated the three-dimensional trajectories of both paws to conserve their relative position in space. We interpret these results as providing unique evidence for the development of functional kinesthetic senses and a capacity to acquire and express motor learning during fetal development.

Specificity of the Learned Motor Response

Several alternative explanations might be offered to account for behavioral changes induced by the interlimb yoke that would not constitute actual motor learning. One obvious possibility is that exposure to limb restraint evokes a general increase in limb activity—a kind of struggling response—that entails an unconditioned increase in vigorous, synchronized kicking. This seems to us unlikely, because CLM increased gradually, not abruptly, during yoke training. But the possibility should be ruled out empirically.

As described above, the distinctive response of fetal rats to hindlimb yoke training is to express an increase in hindlimb CLM. But no concomitant increase in forelimb activity or CLM was noted. To examine the specificity of the training response, and also to scrutinize the "struggling" hypothesis, two additional experiments were conducted to expose fetal subjects to interlimb training of different pairwise combinations of limbs. In the first experiment, the interlimb yoke was attached to the wrists of both forepaws

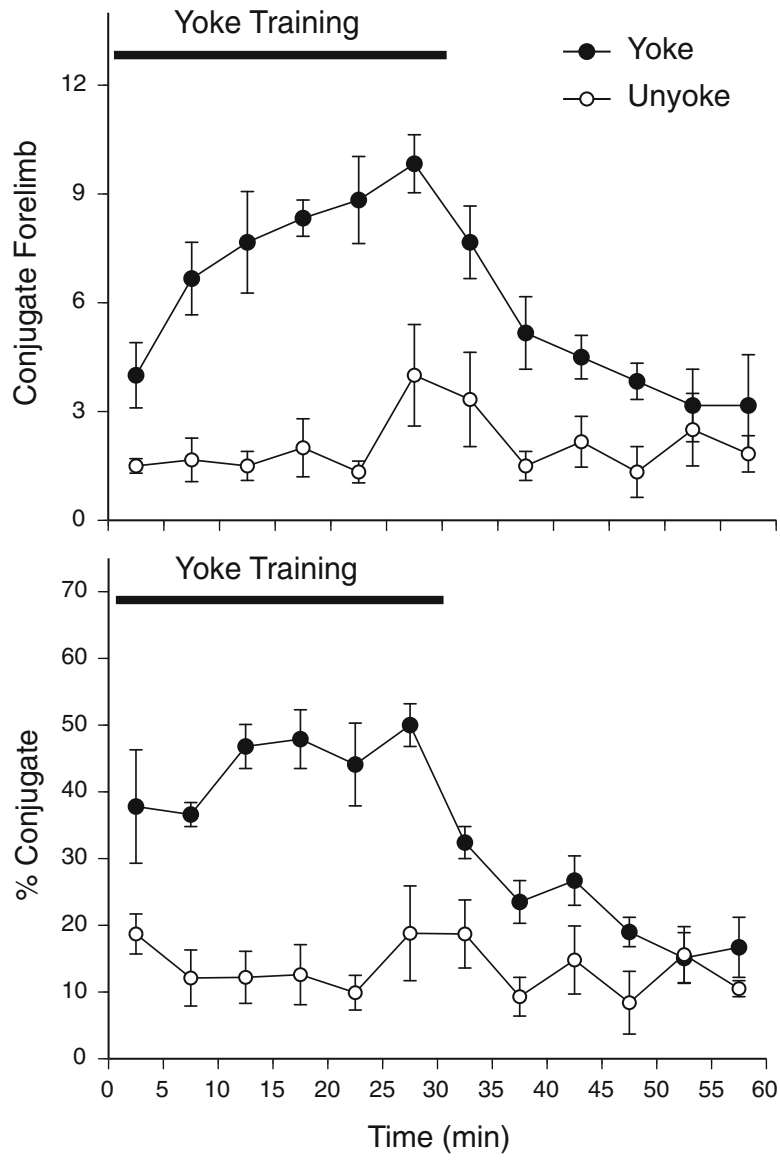

Fig. 3.5 Changes in conjugate movement of forelimbs during and after forelimb yoke training. Conjugate movements are shown as frequency counts (*top*) and as a percentage of overall forelimb activity (*bottom*). *Points* show means; *vertical lines* depict SEM

during the Training Period. In the second experiment, the yoke was attached to the wrist of one forelimb and the ankle of the ipsilateral hindlimb.

As before, the interlimb yoke consisted of an 8-mm length of suture thread. Yoked fetuses were exposed to a 30-min period of yoke training, after which the yoke was divided and subjects observed for an additional 30-min Test Period. Forelimb activity did not differ between Yoked and Unyoked subjects during any part of the experimental session. Hindlimb activity also was not affected by yoke training of the forelimbs. However, CLM of the forelimbs changed significantly during the Training Period in Yoked subjects (Fig. 3.5, top). As in the hindlimb experiments, forelimb CLM increased gradually during yoke training, reaching a peak just before division of the yoke. CLM continued to be expressed at levels several times that of Unyoked controls through most of the Test Period, after the limb constraint created by the yoke was eliminated. Only in the last 5-min interval of the Test Period did forelimb CLM return to control levels. A similar pattern of results was found when CLM was expressed as a percentage

of overall forelimb activity: %CLM increased relative to Unyoked controls during the second half of the Training Period and persisted above control levels for the first 10 min of the Test Period (Fig. 3.5, bottom).

Yoke training of homolateral limbs also resulted in systematic changes in interlimb coordination. As in previous experiments, the interlimb yoke was attached at the beginning of the 30-min Training Period and divided at the onset of the 30-min Test Period. To accommodate the greater distance between ipsilateral forelimb and hindlimb, the length of the yoke was increased to 20 mm. Yoke training was restricted to just one side of the body, which was designated the ipsilateral pair. Half of the fetal subjects were trained on the right forelimb-hindlimb pair, and half on the left. The pair of limbs on the side opposite the interlimb yoke was designated the contralateral pair, and constituted a within-subject control comparison. Separate analyses were conducted to examine changes in activity and CLM of the ipsilateral and contralateral limb pairs.

Yoke training resulted in modest changes in overall activity of the forelimbs. The overall effect was for yoke training to reduce activity in the ipsilateral forelimb during the first 10 min of training, and to increase activity in the contralateral forelimb in the last 20 min of training, compared to Unyoked subjects. Hindlimb activity also was affected by homolateral yoke training. As in forelimbs, activity was depressed in the ipsilateral hindlimb during the first 10 min of yoke training and markedly elevated in the contralateral hindlimb for the duration of the Training Period relative to Unyoked subjects. This pattern of effects on overall limb activity is very similar to the effects produced by attaching a small weight to one forelimb in neonatal rats (Brumley & Robinson, 2013). Unilateral weighting of a limb appears to dampen activity in the weighted limb, but results in a compensatory increase in activity in the unweighted limb. Similar findings have been reported following unilateral limb weighting in human infants (Thelen, Skala, & Kelso, 1987; Ulrich, Ulrich, Angulo-Kinzler, & Chapman, 1997; Vaal, van Soest, & Hopkins, 2000).

Although homolateral yoke training produced modest effects on limb activity, the incidence of CLM was markedly and differentially altered by yoke training. CLM was scored following conventions devised for forelimb pairs and hindlimb pairs: a conjugate movement was judged to occur when a forelimb and hindlimb on the same side of the body initiated movement at the same time and followed parallel trajectories. In other words, CLM was only scored when both limbs flexed or extended together, not when one synchronously flexed as the other extended. This pattern of ipsilateral limb movement was extremely uncommon in Unyoked subjects, and was in fact observed only once during the Training Period in an ipsilateral limb pair and twice during training in a contralateral pair. However, CLM in the ipsilateral limb pair increased markedly in Yoked subjects. CLM also increased initially in the contralateral limb pair during the first 10 min of yoke training, then dropped to near control levels by the end of the Training Period (Fig. 3.6, top). The increase in CLM in the contralateral pair appeared to be due to the differential activity of the ipsilateral and contralateral limbs. When CLM was expressed as a percentage of overall activity in their respective limb pair, they remained very pronounced in ipsilateral limbs, but diminished to near control levels in the contralateral limbs (Fig. 3.6, bottom).

The findings from the forelimb and homolateral limb training experiments are consistent with a motor learning interpretation, and offer no support for the argument that the interlimb yoke evokes an unconditioned response that involves synchronized limb activity. During forelimb yoke training, only the forelimbs express an increase in CLM; the hindlimbs remain unaffected. During homolateral yoke training, CLM are markedly elevated in the forelimb-hindlimb pair that experiences the interlimb yoke, but are virtually absent in the contralateral limb pair. CLM is not a common pattern of interlimb coordination in the rat fetus, particularly in forelimb-hindlimb pairs, and its expression in response to yoke training satisfies many of the defining features one would expect of true motor learning.

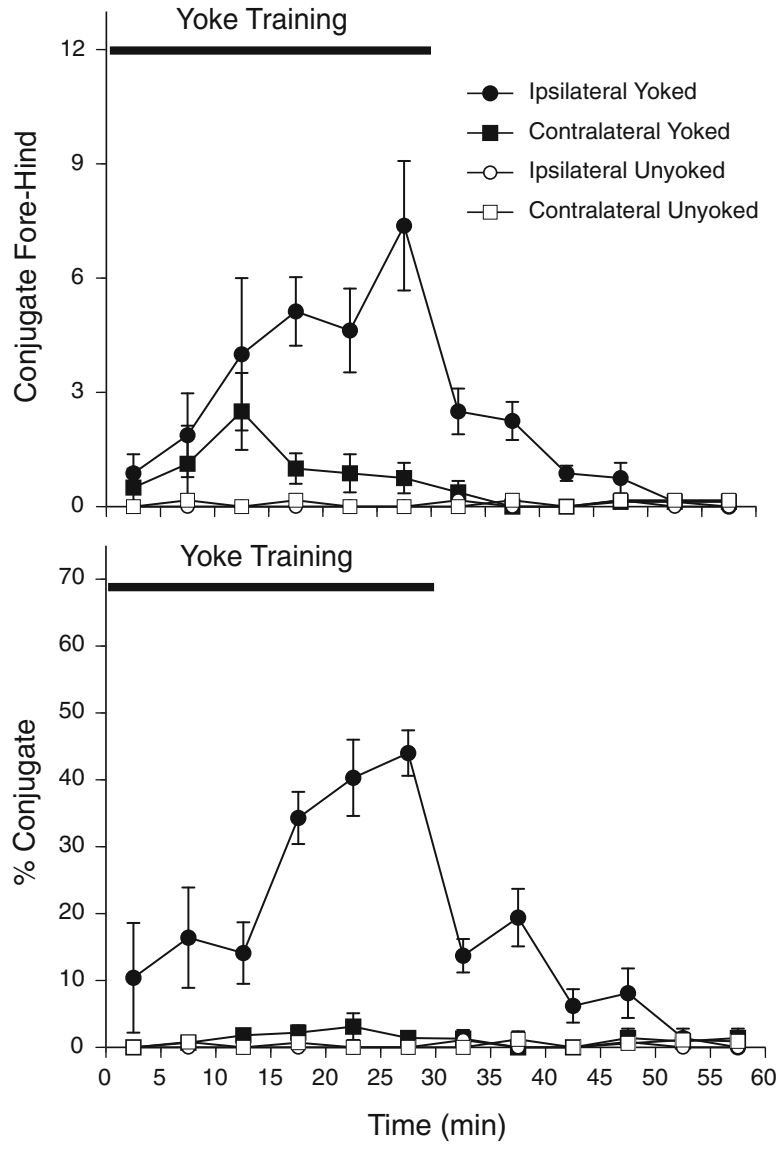

Fig. 3.6 Changes in conjugate movement of forelimb-hindlimb pairs during and after homolateral yoke training. The yoke was applied to one forelimb and hindlimb (Ipsilateral), and the other pair was unmanipulated during training (Contralateral). Unyoked subjects were fitted with a yoke that was immediately divided. Conjugate movements are shown as frequency counts (*top*) and as a percentage of overall limb activity (*bottom*). *Points* show means; *vertical lines* depict SEM

Prenatal Development of Yoke Motor Learning

Motor development in the rat occurs in the context of dramatic changes in physical growth and the immediate environment, both intrauterine and postnatal. These changes in the body and the fetal milieu pose substantial challenges to the fetus's ability to generate and sustain coordinated action as well as opportunities for motor experience (Brumley & Robinson, 2010; Robinson & Kleven, 2005; Robinson & Méndez-Gallardo, 2010; Ronca et al., 1994; Ronca, Lamkin, & Alberts, 1993). The period of late gestation in the rat—from E18 to E21—is a time of rapid growth and development. During this period, the fetus grows at a nearly exponential rate, with body length increasing 182 % (20.5–37.4 mm) and body mass 344 % (1.6–5.5 g) (Robinson, 1989; Robinson & Smotherman, 1992c). The earliest movements are expressed only 2 days earlier, on E16, and the first responsiveness to exteroceptive sensory stimuli 1 day later. Overall motor activity

rises to a peak by E18 (Narayanan et al., 1971; Robinson, 1989; Smotherman & Robinson, 1986). Over the next 3 days (E18–21), fetal movements show increasing temporal and spatial organization (Kleven et al., 2004; Robinson, 1989; Robinson, Blumberg, Lane, & Kreber, 2000; Robinson & Smotherman, 1987), as fetuses begin to express a repertoire of coordinated action that foreshadows functional behavior of the neonate and adult (Robinson & Brumley, 2005; Robinson & Smotherman, 1992a; Smotherman & Robinson, 1987). Coordinated motor patterns that first appear at this time include elements of suckling (Robinson et al., 1992; Robinson & Smotherman, 1992a), grooming (Robinson & Smotherman, 1991), head orientation (Robinson et al., 1992), maternal-infant interaction (Smotherman & Robinson, 1988b), postural control (Ronca & Alberts, 1994), and locomotion (Bekoff & Lau, 1980; Brumley & Robinson, 2005). In the rat fetus, the short span from E18–E21 represents a crucial period in which the foundations of motor control and coordination are first established.

Our early experiments with motor learning in the rat fetus all were conducted on gestational age E20, 2 days before birth, when nearly all of the fetal repertoire described above is in place. Can younger fetuses also respond to kinesthetic feedback? Could motor learning contribute to the dramatic early development of the motor system that takes place after E18? To begin to address these questions, the yoke-training paradigm was adapted for application to rat fetuses as young as E18 (Robinson et al., 2008). Fetuses were prepared for observation in the same way, but the interlimb yoke was adjusted to differences in fetal body dimensions: E18: 3 mm; E19: 5 mm; E20: 8 mm; E21: 10 mm. To enforce these dimensions, the yoke was modified to include silk suture threaded through polyethylene tubing cut to the specified length, creating loops at either end of the tubing to be slipped over the feet of the subject. A final modification of the protocol was to add a 5-min baseline period to the beginning of the experimental session, before the interlimb yoke was attached at the ankles.

At the earliest age (E18), fetuses showed no discernible response to hindlimb yoke training. Yoked subjects did not differ from Unyoked controls in the frequency of forelimb or hindlimb movements or the incidence of hindlimb CLM. One day later, E19 fetuses did not alter overall activity of forelimbs or hindlimbs during yoke training, but did express modestly elevated levels of CLM during the last 20 min of the Training Period and the first 10 min of the Test Period (Fig. 3.7). The performance of E20 fetuses replicated findings from earlier experiments, with pronounced increases in hindlimb CLM as early as the second 5-min interval of Training, increasing to a peak nearly 17-times greater than during baseline, before the interlimb yoke was attached. Moreover, CLM persisted above Unyoked levels throughout the 30-min Test Period. But there was no difference between Yoked and Unyoked subjects in the frequency of hindlimb or forelimb movements during the experimental session. At the oldest age, E21 fetuses showed the most pronounced response to hindlimb yoke training, reaching levels 30 % greater than on E20. CLM continued to be expressed at high levels throughout the Test Period, declining to only 50 % of peak levels by the end of the session (Fig. 3.8). At all ages, CLM was virtually absent in Unyoked subjects. Overall, these age-related changes in response to interlimb yoke training suggested that fetuses become sensitive to movement-produced kinesthesia as early as E19, with responsiveness and persistence of effects increasing over successive ages.

We may infer from the relatively weak response of fetuses on E19 that they lack the necessary neural mechanisms to either detect changes in limb position and the impediment to limb movement created by the interlimb yoke, or the mechanisms to modify motor output in response to that information. Afferent information may be provided from a variety of sources: muscle spindles, tendon organs, joint angle sensors, or cutaneous receptors that provide movement feedback when the skin is stretched or relaxed. As mentioned above, anatomical and physiological evidence suggest that muscle spindles, at least, can

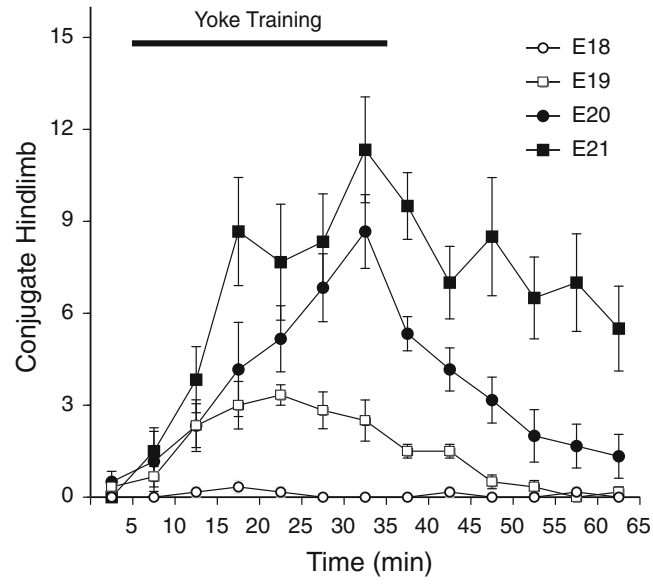

Fig. 3.7 Changes in conjugate hindlimb movements during and after yoke training at four gestational ages. Only Yoked subjects are shown; Unyoked subjects showed very low rates of conjugate activity at all ages. *Points* show means; *vertical lines* depict SEM

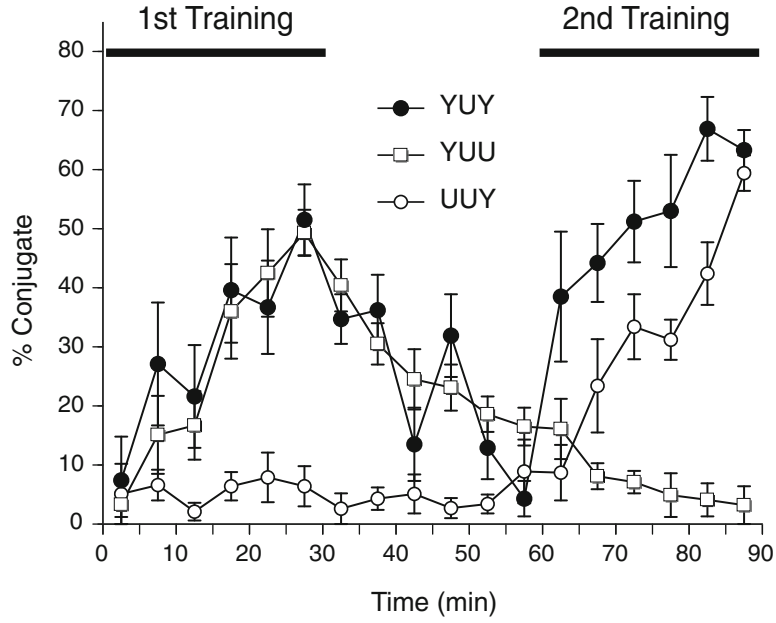

Fig. 3.8 Changes in conjugate hindlimb movements, expressed as percentage of overall hindlimb activity, during two repeated Training Periods. E20 fetal subjects received interlimb yoke training during the first 30 min of the session only (YUU), during the last 30 min of the session only (UUY), or during both the first and last 30 min of the session (YUY). Note that fetuses exposed to the interlimb yoke during a second Training Period showed more rapid elevation of CLM. *Points* show means; *vertical lines* depict SEM

produce functional afferent feedback by E19 (Fitzgerald, 1987; Kucera et al., 1989). Cutaneous responsiveness also is well documented in fetuses as early as E17 (Narayanan et al., 1971; Smotherman & Robinson, 1988c), including sensitivity to mechanical stimuli that mimic conditions encountered during maternal behavior and labor (Robinson et al., 1992; Ronca et al., 1993; Ronca & Alberts, 1994). Feedback produced by cutaneous afferents has been implicated in certain biomechanical effects on chick motility (Bradley & Sebelski, 2000) and in modulating the expression of coordinated action in the rat fetus (Robinson & Smotherman, 1994; Smotherman & Robinson, 1989). Given this evidence for fetal responsiveness to various sources of kinesthetic information, it is unclear whether sense organs in muscles, skin, or other parts of

the musculoskeletal system are responsible for enabling yoke motor learning, or whether the same combination of kinesthetic sensors are contributing to motor learning at all prenatal ages.

Savings and Motor Memory

In most traditional learning paradigms, the effects of repeated practice or training are assessed using probe trials interspersed with training trials or after the conclusion of training. For motor learning to influence behavioral development, it must exert effects that persist beyond the immediate conditions that reward behavioral adaptation. Retention refers to the persistence of effects after the termination of training, and memory refers to the underlying mechanism or process that supports retention of learned changes in behavior. Memory is seldom measured directly, but only inferred from behavior expressed after some delay, either as recall, when the behavior is immediately expressed upon reexposure to similar conditions, or savings, when the rate of learning is accelerated in later learning situations. For interlimb yoke training to represent motor learning, there must be some evidence of persistent behavioral effects. There must be evidence of memory.

An initial experiment designed to test the memory hypothesis was conducted in a single experimental session with E20 rat fetuses (Robinson, 2005). The 90-min session comprised a first Training Period of 30 min, an intervening Test Period lasting 30 min, and a second Training Period, also of 30 min. Three groups of fetal subjects were exposed to hindlimb yoke training in this study. The first group, designated YUU, received yoke training during the first Training Period, but remained Unyoked during the second Training Period. The second group, designated UUY, received the opposite treatment, remaining Unyoked during the first Training and Yoked during the second. The third group—YUY—received yoke training during both Training Periods. All three groups remained Unyoked during the Test Period. Our expectation was that fetuses exposed to a second period of yoke training would either show spontaneous recall, immediately expressing elevated levels of CLM, or savings, in which they showed accelerated rate of change in CLM or expressed higher peak levels of CLM during the second Training.

The results from this experiment are summarized in Fig. 3.8. Subjects in all three groups responded appropriately to yoke training. In both the YUU and YUY groups, CLM increased gradually during the first Training Period, with CLM significantly elevated above Unyoked subjects (the UUY group) by the third 5-min interval of training, and ultimately reaching peak levels more than ninefold greater than controls. These changes in conjugate activity were evident in both the frequency of CLM and CLM expressed as a percentage of overall hindlimb activity.

During the Test Period, after the yoke was cut, the rate of CLM diminished as observed in previous experiments. CLM continued to be expressed at levels higher than Unyoked controls (the UUY group) until the last 5-min interval of the Test Period, when only the YUU group remained elevated (The YUU group returned to control levels in the next interval, the first 5 min of the second Training Period.) Thus, in both the YUY group and the UUY group, CLM occurred at very low rates immediately before the onset of the second Training Period.

During the second Training, CLM rapidly increased in both Yoked groups. However, it increased at a more rapid rate among fetal subjects that had experienced yoke training earlier (the YUY group). CLM was elevated in the YUY group during the first two 5-min intervals of training, in contrast to the UUY group, which began to show significant increases in CLM only in the third 5-min interval of training. (This pattern, of course, was exactly similar to that expressed by the other two groups in the first Training Period.) Differences in the rates of CLM during the second Training Period were clearly evident in both frequency counts and as a percentage of overall hindlimb activity. However, although CLM increased quickly in the YUY group, it was not expressed at peak levels until the last 10 min of the Training Period, when it accounted for more than 65 % of all hindlimb movements (Fig. 3.8).

Changes in the rate of acquisition of motor learning between an initial and a subsequent training session are consistent with the interpretation that prior experience with the interlimb yoke accelerates the rate of learning during a second exposure to yoke training. This acceleration appears to represent savings, which implies a memory-like process that preserves information about prior limb training even after overt behavior has returned to pretraining levels. It would be desirable to extend the delay between the first and second Training Periods to explore the nature and stability of this memory process. Unfortunately, methods used to prepare rodent fetuses for direct behavioral observation are difficult to maintain in excess of a few hours and preclude longitudinal study. We have, however, applied the same methods of interlimb yoke training to neonatal rats tested on P1, 24-h after birth. The responsiveness of newborn rats to yoke training is virtually indistinguishable from that of near-term fetuses (Brumley & Robinson, 2010). Moreover, rat pups can be exposed to yoke training over successive days in a fashion that is not possible with fetuses. Postnatal experiments involving repeated yoke training have confirmed that this form of motor learning can persist on a scale of days, not merely hours. Pups exposed to a second yoke training session 24-h after an initial training session showed savings that were very similar to the savings reported above, after only a 30-min delay (Robinson, Woller, Khetarpal, Fromm, & Brumley, 2004). Retention of information from motor experience on this time scale is sufficient to exert an impact on developmental processes, suggesting that motor learning may contribute to normal motor development in utero.

Implications of Yoke Motor Learning for Fetal Behavioral Development

The experiments described above represent only a few of the studies that my laboratory has conducted to explore the potential for motor learning in the fetal and neonatal rat (Brumley & Robinson, 2010; Robinson, 2015). The interlimb yoke training paradigm is relatively simple to apply to both fetal and neonatal subjects across a range of species, including humans. Indeed, it appears to have been independently discovered (invented) by three different laboratories working with developing rabbits (Viala, Viala, & Fayein, 1986), human infants (Thelen, 1994), and fetal rats (Robinson & Smotherman, 1994).

In rabbits, normal locomotor development entails a transition in gait patterns during the first 3 weeks after birth. Newborn rabbits exhibit a crawling gait, similar to walking, that involves a typical pattern of alternation between left and right limbs within a girdle. Between 10 and 20 days after birth, however, young rabbits experience marked changes in morphology as the hindlegs and feet grow disproportionately compared to the forelimbs. During this time, rabbits undergo a transformation in their predominant gait, which resolves as a half-bound, in which the forelegs step forward alternately and the hindlegs follow in a synchronous hop. This pattern of transition from an antiphase pattern of interlimb coordination in infancy to an in-phase coordination of the hindlimbs in adulthood is characteristic of the development of young animals that exhibit hopping or ricochetal locomotion as adults, including gerbils (*Gerbillus dasyurus*) (Blumberg-Feldman & Eilam, 1995), jirds (*Meriones tristrami*) (Eilam, 1997), kangaroo rats (*Dipodomys stephensi*) (Lackey, 1967), (*Dipodomys ordi*) (personal observation), and jerboas (*Jaculus orientalis*) (Eilam & Shefer, 1997).

Is the transition from crawling to hopping dictated by maturation of pattern generating circuitry in the central nervous system of rabbits, or is it influenced by feedback from changing anatomy during development? This question was addressed experimentally by Viala and colleagues using a procedure very similar to our interlimb yoke-training paradigm. In this study, newborn rabbits were prepared with a mid-thoracic spinal transection two days after birth. Without further training, rabbits spinalized as infants can express hindlimb locomotor behavior as adults if care is taken to prevent spasticity and muscle atrophy. The behavior expressed by spinalized rabbits is atypical,

however, exhibiting both in-phase and antiphase patterns of interlimb coordination (Fayein & Viala, 1976). To determine whether motor experience could bias their development toward one pattern or the other, young rabbits were placed on a motorized "bicycle", which permitted either in-phase or antiphase hindleg movement, in six 15-min training sessions each day over a 20-day period. At the end of this period, rabbits that received only antiphase bicycle training expressed pure hopping gaits, rabbits that received antiphase and in-phase training on successive trials showed alternation about 50 % of the time, and rabbits that received only in-phase training expressed alternated stepping about 75 % of the time (Viala et al., 1986). These findings strongly suggest that experience during the few weeks after birth serves to shape spinal circuits that govern species-typical interlimb coordination.

The interlimb yoke training used in our laboratory is even more closely mirrored in an experiment conducted by Thelen (1994). Thelen used a conjugate reinforcement paradigm to promote leg kicking in 3-month-old infants, following procedures well established by Rovee-Collier and other developmental researchers (Kraebel, Fable, & Gerhardstein, 2004; Rovee & Rovee, 1969; Rovee-Collier, Hayne, & Colombo, 2001; Rovee-Collier, Morrongiello, Aron, & Kupersmidt, 1978). In this paradigm, a ribbon is attached to one leg of the supine infant, and the other end tied to a mobile suspended overhead. The ribbon creates a physical linkage between the infant's leg and the mobile, such that kicks by the infant cause the mobile to shake and jiggle. Infants find this highly rewarding, and quickly learn the contingency to kick in order to see the mobile move. Thelen modified the standard paradigm by attaching a length of elastic to both of the infant's ankles. Over 10 min of training with the overhead mobile and interlimb elastic, infants began to kick in a conjugate pattern, synchronously extending and flexing both legs, which resulted in more vigorous shaking of the mobile. Similar efforts to constrain limb movements have produced similar effects on infant motor behavior (Angulo-Kinzler, 2001; Angulo-Kinzler & Horn, 2001; Chen, Fetters, Holt, & Saltzman, 2002).

Like Viala's spinalized rabbits and the rat fetuses in our laboratory, these changes in interlimb coordination can be viewed as adaptive, wherein the infant adjusts its motor behavior to compensate or overcome a constraint on limb coordination. However, our yoke training involves only self-generated movement, not motorized training (unlike the Viala study) and involves no explicit reinforcement to encourage behavior (unlike the Thelen study). Thus, only the intrinsic characteristics of the interlimb yoke are necessary for fetuses to adaptively modify their motor coordination.

The ability of fetuses to do this is no mean feat (pun intended). Yoke motor learning implies that the fetal rat must (a) detect the presence of the interlimb yoke, (b) generate kinesthetic signals that distinguish between independent and yoked movements, (c) discriminate between these different patterns of kinesthetic feedback, and (d) modify motor output to selectively favor paths and velocities of movement of each leg to coordinate the 3-D positions of two working points (in this case, the tips of both hindpaws). Presumably, these adaptive changes in motor commands minimize proprioceptive error signals, resulting in altered patterns of interlimb coordination expressed during spontaneous motor activity. Even after contingencies that promoted behavioral change are removed, some form of motor memory persists, probably in structurally altered spinal motor circuits (Robinson, 2015). If fetuses can demonstrate this level of sophistication in adaptively modifying their motor behavior in an experimental setting, then they may be equally responsive to contingencies experienced in utero during the formative development of their motor system (Brumley & Robinson, 2010).

In the last few days of gestation, spontaneous movement by the rat fetus changes in frequency and increases in organization (Kleven et al., 2004; Robinson, 1989; Robinson et al., 2000; Robinson & Smotherman, 1987, 1992d). These developmental changes in motor behavior are concomitant with changes in the intrauterine environment and the fetus's relationship to it (Brumley & Robinson, 2010; Robinson & Méndez-Gallardo, 2010). Amniotic fluid volume diminishes sharply

during the last 2 days of gestation in rats and other rodents (Robinson, 1989; Robinson & Brumley, 2005), at the same time that fetal growth has greatly exacerbated problems of physical constraint in utero. Neural mechanisms that generate and modulate motor behavior, including interlimb coordination, undergo dramatic development during this same span of time (Clarac, Brocard, & Vinay, 2004; Kudo, Nishimaru, & Nakayama, 2004; Ozaki, Yamada, Iizuka, Nishimaru, & Kudo, 1996; Vinay, Pearlstein, & Clarac, 2010). Many of the neural resources that regulate spontaneous activity also are shared with mechanisms of voluntary motor control, including locomotion (Bekoff, 1992; Bradley, Solanki, & Zhao, 2005; Brumley & Robinson, 2010). The capacity for prenatal motor learning exists in the context of these associated changes in behavior, neural development, and the intrauterine environment. Is it, then, unreasonable to suggest that learning contributes to the development of motor behavior before birth?

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Fetal Behavioral Development and Brain Growth in Chimpanzees Versus Humans: A View from Studies with 4D Ultrasonography

4

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Abstract

This chapter initially describes how to perceive the relationship between individual development and evolution, then examines the essential nature of heterochronic evolution in humans and chimpanzees, the closest relative of humans, by comparing their fetal behavioral and brain development. We discuss the results of our recent studies on human and chimpanzee fetuses by using four-dimensional ultrasonography. Results showed that the growth velocity of the brain volumes of chimpanzee fetuses does not accelerate during late pregnancy, whereas that of human fetuses does accelerate through late pregnancy. Additional analysis and findings show that the timing of cessation or deceleration in the increase of growth velocity of brain volume among species crucial to clarify how much earlier human infants are born and how retarded is the development of their postural reactions is. Accumulated data suggest that further verification of temporally modified growth and development among species will help us to understand the effect of individual development on the evolution of human behavior.

Keywords

Growth velocity of the brain volume • Postural reaction • Neonate • Fetus • Anticipatory thumb sucking • Finger movement

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Introduction

The mind is a product of evolution as well as physical morphology and its functions. Based on this understanding, advances have been made in comparative developmental studies on cognition and behavior in various animal species. This has led to the possibility of demonstrating the course of evolution of the human mind through the diversity and commonality of the "mind." Particularly, since humans belong to the order of primates, comparative studies among primate species are an essential approach to advancing our knowledge of the origins of human nature. Thus, researchers have been trying to understand the evolution of the human mind by comparing various aspects of development among primates (Bjorklund & Pellegrini, 2002). This chapter initially describes how we perceive the relationship between individual development and evolution, and then examines the essential nature of heterochronic alterations of behavioral development in humans when compared with chimpanzees, by reviewing studies of fetal behavioral development and brain growth in our laboratories.

Evolution of Developmental Systems

The Theory of Evolution of New Behavioral Phenotypes

Mainstream modern biology has defined evolution as "changes in genotype frequencies in a group." Changes occur in genes involved in the expression of certain behaviors and morphology; new genes emerge, and if this extends further, genotype frequencies in the group will change. Even in the absence of major changes such as the emergence of new species, evolution is said to occur when there are changes in the frequency of genotypes associated with certain traits in a group. What causes these alterations in genotype frequencies has long interested scientists. Natural selection has been assumed to be the most predominant mechanism. In addition to staying alive, living organisms give birth to a large number of offspring, which may carry genetic mutations. If some of the mutations are favorable for survival and reproduction, such mutations will spread through generations. In other words, changes in genotype frequencies will occur within the population. Subsequently, if certain genetic variations consistently express adaptive traits favorable to survival and reproduction in their environment, the relevant mutations will remain selected and will have the propensity to be inherited by the next generation. However, the reality is certainly not so simple.

Apart from a single gene expressing a specific trait, various mutations and changes also occur within the environment to which the individual needs to adapt. Most importantly, bidirectional interactions can always occur within each level and between different levels of elements composing an individual, namely, genes, cells, tissues, organs, organ systems, living organisms, and the environment (Gottlieb, 1992). In other words, differences between acquired traits are controlled not only by genetic differences, but also by the process of development of each individual, which begins with their fecundation and conception, and includes the effects of genes. As a result, the genes of those individuals who were successful in reproduction are inherited. However, this does not guarantee survival and successful reproduction in the next generation. The survival and reproductive potential of the individual is primarily probabilistic, and results from the individual's own existence and actions, which are in turn involved in a changing ecological and social environment, and in a process of dynamic transformation of the developing body and mind. It is through this unpredictable process (i.e., through individual development) that genetic mutations are inherited by the next generation, along with the immediate physical environment, which serves as a habitat for the species, as well as the social environment (Lickliter & Berry, 1990; Oyama, 2000).

In an attempt to reconsider the relationship between individual development and evolution on the basis of the understanding of epigenetic development described above, Gottlieb (1992, 2002, 2007) focused his attention on a phenomenon called "genetic assimilation." This refers to

the fact that traits that newly emerge during the developmental process of an individual under certain environmental conditions are repeatedly manifested throughout generations, and over time, and continue to appear even when the environment changes from its original condition. He assumed that new behavior patterns originating from the development of an individual were inherited through generations, and categorized the mechanisms inducing changes in genotype frequencies, the mechanisms by which evolution occurs, into three stages. This was called the "theory of evolution of new behavioral phenotypes" (Bjorklund & Pellegrini, 2002; Gottlieb, 1992, 2002, 2007; Pappini, 2002). The first stage consists of changes in development and behavior; the second stage consists of morphological or physiological changes, and, the third stage consists of genetic changes. In the first stage, new behavioral phenotypes are inherited through generations, resulting in a new relationship with the environment. In the second stage, the new relationship with the environment causes physiological or morphological changes, and leads to the possibility of mutations in somatic cells and potential changes in regulatory genes. However, at this stage, changes in structural genes do not occur. In other words, the final stage of evolution involves the occurrence of changes in the genotype frequency of structural genes in a population. The evolutionary process is perceived to include the morphological or physiological changes occurring prior to the changes in genotype frequencies as well as the behavioral changes occurring prior to morphological or physiological changes. In the case of changes in the environment, or in the case of individuals who alter their own behavior to explore new environments, minor genetic changes occur, or genes that had previously not been expressed are activated. It has been pointed out that such phenotypic changes might lead to reproductive isolation, and alter the genetic makeup of the species as a result. Even if evolution is achieved through the mechanism known as natural selection, this theory provides an answer to the issue of how the selected new traits first occur. In other words, even without depending on genetic mutations, genetic drift, or the inheritance of acquired characteristics, new behavioral phenotypes have been shown to occur as a result of individual development. Those new behavioral phenotypes are then maintained across generations, and become eligible for selection in the course of evolution.

Heterochronic Alterations

From the perspective of the relationship between individual development and evolution, the hypothesis that the beginning of evolution is marked by the emergence of a new behavioral pattern in individual development and alterations in the developmental process, as is seen in Gottlieb's theory of evolution, can be traced back to Haeckel's well-known theory of recapitulation (Haeckel, 1879), according to which "ontogeny recapitulates phylogeny." According to this theory, ontogeny is a process in which phylogeny, namely, the evolutionary path that the species has followed, is carried out repeatedly. Neomorphs, which are the products of evolution, are added at the final stages of ontogeny. As is generally known, a comparison of the ontogenies of various species has shown that this is an unverifiable hypothesis. In other words, phylogeny is not repeated in ontogeny. Instead, ontogeny and individual development proceed by changing phylogeny. Such concepts have been passed down, along with the focus on a phenomenon called "heterochrony" (Gould, 1977, McNamara, 1997).

Heterochrony refers primarily to a change in the relative speed and timing of the expression of traits that were previously present in ancestors. As an essential element in the mechanism of the evolution of traits, it has thus far been studied most often in relation to the evolution of morphological characteristics. The variations in timing and speed are assumed to comprise eight main processes, including neoteny (pedomorphosis) (Parker & McKinney, 1999). The morphology of the skull, for example, has been considered to be representative of the characteristics of human neoteny. However, a study conducted by Shea (1989) has emphasized the possibility that the differences between the specific developmental

patterns of chimpanzees and bonobos are due to the effects of the excessive growth of the tongue and larynx. The articulatory organs are composed of the tongue and the larynx, and in humans, the larynx descends along the back of the neck during infancy, while the shape of the tongue becomes spherical. This phenomenon of the descent of the larynx is necessary for the development of speech, and is seen in humans, but not in other primates, including chimpanzees. However, according to a recent study, the phenomenon of laryngeal descent seems to have emerged in two phases in the process of primate evolution (Nishimura, Mikami, Suzuki, & Matsuzawa, 2003). First, the common ancestors of the hominoids (gibbons, great apes, and humans) acquired changes consisting of a descent of the larynx relative to the hyoid bone during their growth. This was not found in their common ancestors with the Old World monkeys. Subsequently, after humans diverged from the common ancestor that they shared with the lineage of chimpanzees, they acquired a change in growth, which is the descent of hyoid bone relative to the skull base and the mandible. This marked the achievement of the phenomenon of laryngeal descent unique to humans.

Heterochrony also can be defined as an essential element in the mechanism of the evolution of behavioral development (Minugh-Purvis & McNamara, 2002). Previous comparative studies on behavioral development have indicated that, in terms of postural control and locomotion, human neonates showed delayed development compared to great apes. However, some aspects of early cognitive development are markedly more advanced in humans than in great apes (Parker & McKinney, 1999; Takeshita, 1999). Regulatory genes are assumed to be involved in the emergence of heterochrony, and their role in the evolutionary mechanism based on the new behavioral phenotypes proposed by Gottlieb can be considered as well. Changes in the speed and timing of behavioral development may occur as a result of an adaptation to environmental changes (Bjorklund & Pellegrini, 2002; Pappini, 2002). New behaviors unique to the species are also likely to emerge as a result of modifications in the developmental processes such as through reorganization and a shift in the timing of many aspects of the development of preexisting behaviors within the species (Langer, 2000).

Humans share the phenotypes of basic characteristics with other primate species, especially chimpanzees, which are genetically the closest to humans. For example, they share movement related to posture, locomotion, and the use of fingers and objects. However, as described above, the speed and timing of development in these areas changed in ways that are characteristically unique to humans: being stable in the supine position from early on, the fine movements by fingers of both hands, object manipulation for tool use or to construct something complex, and a social nature supported by interaction with others. These unique characteristics differentiate human development from that of other species (Takeshita, Myowa-Yamakoshi, & Hirata, 2009). Further, uniquely human connections with caregivers also enable the acquisition of these characteristics. Most likely, differentiation by social cognition and sympathy as well as imagination has enabled humans to experience a qualitatively dramatic developmental evolution. When determining the reason for the development of elements underlying these new behaviors, one must be aware of the heterochronic alterations in human behavioral development compared with chimpanzees, the closest relative, at as early a stage as possible.

Fetal Behavioral Development in Humans and Chimpanzees

How to Be Born

Among primates, humans have an oversized brain, which may necessitate an increased body weight as a source of energy for sustaining it. For early humans who moved to open lands, there have been indications that the overall enlargement of the body might have been a rather important adaptation (Pawlowski, 1998). When humans started to leave the forest and sleep on the ground at night, they needed a suitable body weight and fat in order for their babies to be able to withstand the cold environment.

The necessity for babies with the largest possible head or the largest possible body made the childbearing process different from that of most other primates (Rosenberg & Trevathan, 1995). During childbirth in humans, the fetus, who has reached a size that barely allows him/her to pass through the birth canal, typically rotates his/her head in a direction that allows him/her to pass through more easily, then proceeds forward through the birth canal. Eventually, the fetus turns his/her face to a direction opposite that of the mother and is born with the head held in dorsiflexion. This posture makes it extremely difficult for the mother, who is exhausted from the delivery, to provide assistance to ensure the safety of the baby, whatever posture she adopts. In Old World monkey species such as Japanese monkeys, the baby is born in a posture where the head is bent forward so that his/her face is turned toward the same direction as that of the mother. Therefore, all that the mother has to do is to take the baby in her arms and put him/her on her abdomen while supporting his/her body from the head to the back, using one hand. In contrast, a human baby can be born in two different ways: he/she can "drop by himself/herself and be born without any assistance" or he/she can be born with the assistance of someone other than the mother who "takes the baby out" after which he/she will be taken by the mother in her arms. The enlarged size of human newborns prevents physical contact between the mother and the baby at the time of birth. The newborn is placed in the supine position beside his/her mother and is held in the arms by people other than the mother. This opens the way to opportunities for people other than the mother to get involved with the baby as early as at the time of birth.

Recent research based on the first clear close-up video recordings of three chimpanzee births in captivity revealed, however, that the chimpanzee newborns emerged with an occiput anterior orientation, involving head and body rotation at least after the head had emerged. In addition, the chimpanzee newborns landed on the ground in two of the three cases, without being guided from the birth canal by the mother (Hirata, Fuwa, Sugama, Kusunoki, & Takeshita, 2011). The fact that the human newborn emerges with an occiput anterior orientation has thus far been taken as evidence for the necessity of midwifery in modern humans, but this view needs to be reconsidered. The observation raises the issue of the evolutionary scenario of human birth.

From the next section, we focus on the example of the coordination and distortion of individual development in fetuses between humans and chimpanzees, by reviewing our recent comparative developmental studies.

How Do Human and Nonhuman Fetuses Behave?

We have been conducting comparative developmental studies of fetal behavior in humans and chimpanzees using a four-dimensional diagnostic ultrasound imaging system (4D-US) with the chimpanzee mothers and their fetuses mentioned in the previous section. Recently, this device, which has been widely used in obstetrics and gynecology, has facilitated clear visualization of facial expressions and hand movements of the fetus. We have for the first time revealed that anticipatory thumb sucking, which is found during infancy (Butterworth & Hopkins, 1988), can already be observed during mid-pregnancy in humans (Myowa-Yamakoshi & Takeshita, 2006). When a finger is headed toward the mouth, the mouth opens right before the finger comes in contact with it, and subsequently, the finger enters the open mouth, and the fetus appears to suck its finger. This has been observed after the 20th week of pregnancy in humans. The finger's motion and the opening and shutting of the mouth are coordinated smoothly. Fetuses also have been found to begin showing subtle finger movements and aligning both hands at the same period. From mid to late pregnancy, the human fetus frequently uses his/her hand to touch various regions of his/her own body, including the head, face, mouth, feet, and the other hand. It might be through this behavior that the fetus learns the positional relationship between these respective regions. In addition, it might be through this double touch experience

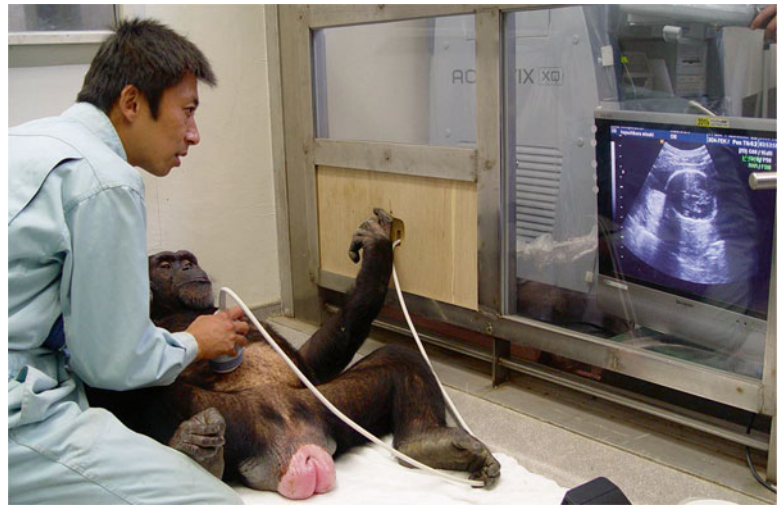

Fig. 4.1 Close relationship between chimpanzee mothers and human researchers enabled examination using 4D-US

that the fetus obtains information on his/her own body and its movements, and starts to perceive his/her own "self" (Rochat, 2001; Rochat & Hespos, 1997; Takeshita, Myowa-Yamakoshi, & Hirata, 2006; Takeshita et al., 2009).

How do these behaviors observed in humans compare to those in fetal chimpanzees? Thus far, we have conducted observations on three chimpanzee fetuses, collecting data from the fifth week of fetal life. Various body movements similar to those of human fetuses have been found and are described below. However, the anticipatory thumb sucking and the alignment of both hands with subtle finger movements, which are seen in humans, have not been confirmed.

The following section briefly summarizes our observations of fetal behavioral development in chimpanzees and compares it with fetal development in humans. Three chimpanzee mothers (Tsubaki—9 years old; Mizuki—11 years old, and, Misaki—9 years old) participated in the study without any use of an anesthetic (see Fig. 4.1 for an example of a chimpanzee mother ultrasound session, in which she was cooperating with her favorite researcher who was searching the images of her fetus). They belonged to the Great Ape Research Institute, Hayashibara Biochemical Laboratories, 952-2, Nu, Tamano, Okayama 706-0316, Japan (Hirata, 2008). Before beginning the first test session it was necessary to familiarize the chimpanzee mothers with the experimental settings in which they were required to be in contact with the gel on the probe. This was made possible because of the close relationship that the chimpanzees shared with one of the researchers, who operated the probe, and several training sessions conducted over about 2 months. The training sessions had the following three stages: (1) the gel was applied on the belly; (2) the probe with the gel was placed in contact with the belly; and, (3) the probe with the gel was then moved over the belly. Each test session lasted for 6–20 min and was repeated two to three times a week.

0–5 Weeks of Age

The fetal sac was first confirmed at 4 weeks of fetal age in Mizuki, who was the first among three chimpanzees to be examined successfully using 4D-US. At this time, however, the embryo could not be observed. The embryo was confirmed only the following week, at 5 weeks of fetal age. The human embryo at 5 weeks of fetal age weighs about 4 g and is a little over 1 cm in size. The fetal sac is about 3 cm. Thus, we showed that a chimpanzee embryo is very similar in size to the human embryo. The length of the fetal sac was 3.1 cm and was of the exact same shape as that in humans. At about 1 cm, the size of the embryo also was similar to that of a human's. From around 3 weeks of fetal age, the parts of the embryo that will become

the arms and legs grow slowly and, at around 5 weeks of fetal age, the limbs begin to develop finger indents in humans. The parts of the face that will form into the eyes, nose, mouth, and ears also develop. However, various parts of the fingers and face cannot yet be observed using 4D-US.

6–10 Weeks of Age

Ultrasound observation of Mizuki and Misaki showed that the development of chimpanzee embryo follows a course similar to that of humans. Mizuki's fetus was first observed using 4D-US at 8 weeks of fetal age. At this point, the fetus was already frequently moving his/her arms and legs to the front, back, left, and right. Figure 4.2 shows the fetus at 9 weeks of fetal age. He/she was actively moving his/her arms and legs, and the arms seemed more mature than the legs. He/she also had a large head, still proportionated to twice the body size. In general, an organism's development progresses in sequence from the head to the tail. At first, the head is large and brain gets more sophisticated earlier than other organs; the arms develop earlier than the legs. Both human and chimpanzee fetuses develop in accordance with this general pattern of development of organisms. Misaki's fetus was examined successfully for the first time by 4D-US at 9 weeks of fetal age. The fetus was visible, wrapped in the amnion, and the placenta was beginning to form. Around 10 weeks of fetal age, Mizuki's fetus had extended the legs considerably and was becoming much more active. The fingers could not yet be distinguished, but the articulating bones of the wrists, elbows, ankles, and knees were visible in the ultrasound images. The eyes and nose also were vaguely visible. At this stage, chimpanzee and human fetuses are essentially of the same size. The crown–rump length (from the top of the head to the rump) was 2.7 cm, and the biparietal diameter (transverse diameter of the head) was 1.3 cm in Mizuki's fetus at 8 weeks of fetal age. At this time, human fetuses have a mean crown–rump length of 3.0 cm and biparietal diameter of 1.3 cm. In humans, mothers begin to show intensified symptoms of morning sickness around this time. Food preferences may change and they may have nausea or vomiting. From around 1 month after impregnation, all three chimpanzee mothers—Tsubaki, Mizuki, and Misaki—began to clearly show different food preferences. Each individual chimpanzee has her own food preferences, but in general, before pregnancy, they often ate sweet fruits such as oranges and apples. After becoming pregnant, all three individuals favored eating foods they were not expected to like, such as white scallions and bell peppers. Although it is not known if the mechanism is the same as morning sickness in humans, chimpanzees resemble humans in the phenomenon whereby pregnancy is followed by major changes in food preferences.

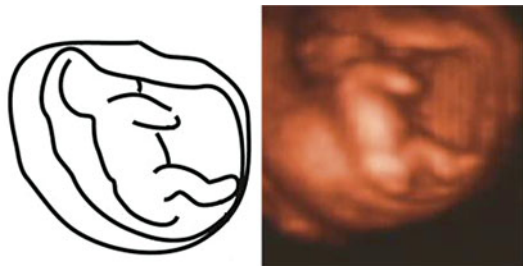

Fig. 4.2 A chimpanzee fetus at 9 weeks of fetal age

11–15 Weeks of Age

Observations of the fetuses of Mizuki and Misaki confirmed that chimpanzees also develop in a manner similar to humans at 11–15 weeks fetal age. First, in both the fetuses of Mizuki and Misaki, the ultrasound screening began showing their umbilical cords at 12 weeks of fetal age. The placenta became larger and more noticeable, and it was inferred that the fetuses received the nutrients from the mother through the umbilical cord. Around the same time, the fetuses' fingers became visible in the ultrasound images. Next, several instances of limb or mouth movements were seen. At 11 weeks of fetal age, we observed a movement in Mizuki's fetus where he/she stretched by bending a knee and kicking the leg diagonally forward. Misaki's fetus was observed opening and closing its hands at 12 week of fetal age. The same motion was first observed at 13 weeks of fetal age in Mizuki's fetus. This fetus

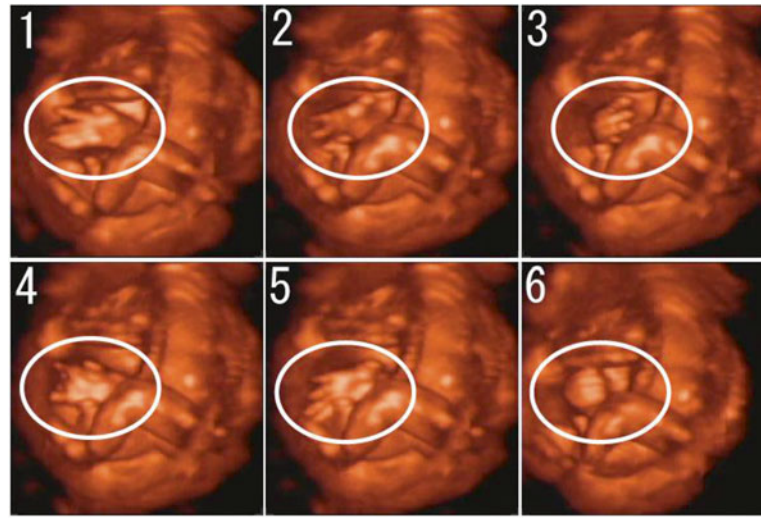

Fig. 4.3 Finger movements by a chimpanzee fetus at 14 weeks of fetal age

did various movements such as opening and closing his/her hands, and subtly moving them while open. When Misaki's fetus was at 14 weeks of fetal age, 4D-US showed brilliantly a series of motions of continuous bending and stretching of its fingers (see illustration of finger movements, Fig. 4.3). Earlier, at 13 weeks of fetal age, we were able to see only that Misaki's fetus was slightly opening and then slightly closing its mouth (see example of mouth opening, Fig. 4.4).

Projections near the external genitalia in the lower half of the body were first observed when Mizuki and Misaki's fetuses were at 13 weeks of fetal age. The formation of the external genitalia also seems to occur at the same time as in humans. However, it is still difficult to ascertain the sex of the chimpanzee fetuses. Male chimpanzees have a penis, but females also have a part of the sexual skin that protrudes. Thus, the presence of projections near the external genitalia area does not make it easy to determine the sex. The size of the human and chimpanzee fetuses at this stage still seems to be substantially the same. However, at this time, the body is growing and assumes different postures; hence, the crown–rump length is not used as an indicator of development in obstetrics and gynecology in humans. Instead, biparietal diameter is now one of the principal indicators of fetal growth. When Mizuki's fetus was at 12 weeks of fetal age, the biparietal diameter was 2.5 cm, and at 14 weeks of fetal age, it was 3.3 cm. The mean biparietal diameter in human

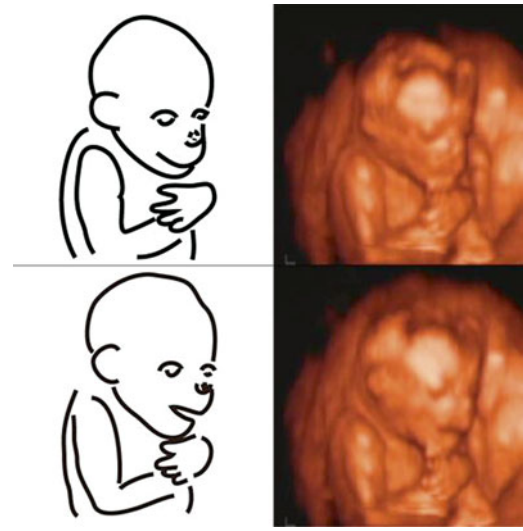

Fig. 4.4 Mouth opening by a chimpanzee fetus at 13 weeks of fetal age

fetuses is 2.6 cm at 12 weeks of fetal age, and 3.3 cm at 14 weeks of fetal age. This coincides with the values of Mizuki's fetus.

16–22 Weeks of Age

At 16 weeks of fetal age, 4D-US showed that Misaki's fetus had brought both hands and both legs to the front of the face and the top of the head (see illustration in Fig. 4.5). Later, the same posture was frequently observed in both Misaki's and Mizuki's fetuses. Differences in body

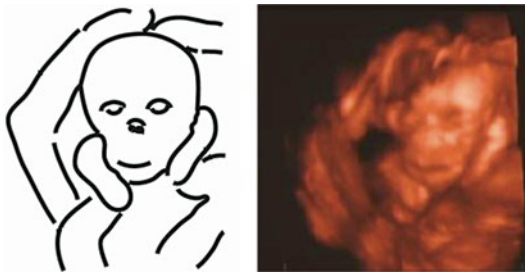

Fig. 4.5 Face and limbs of a chimpanzee fetus at 16 weeks of fetal age

construction between humans and chimpanzees emerge already at this stage. Humans are bipedal walkers. Therefore, the legs are seen to extend straight down from the body. By contrast, chimpanzees are essentially quadrupedal walkers. Their legs take a shape that extends forward relative to the body. Even chimpanzee fetuses have feet that extend in front of the body, so it is probably easier to assume a posture where the legs are brought to the front of the face. A state where both hands and both feet were extended in front of the mouth and appeared to be in contact with each other were frequently observed in both Mizuki's and Misaki's fetuses.

At 19 weeks of fetal age, ultrasound images clearly showed that the chin had a beard in Mizuki's fetus. This is the same period when the human fetus grows hair on the head. The extent of growth in the size of the body gradually begins to differ in pace between humans and chimpanzees. The biparietal diameters of Mizuki's fetus were 3.7 cm at 16 weeks of fetal age, 4.1 cm at 18 weeks of fetal age, and 4.4 cm at 20 weeks of fetal age. The corresponding diameters of a human fetus are 4.2 cm at 16 weeks of fetal age, 4.7 cm at 18 weeks of fetal age, and 5.4 cm at 20 weeks of fetal age. Chimpanzees have a slightly smaller biparietal diameter than humans, and this difference gradually widens.

21 Weeks of Age to Birth

We first observed Misaki's fetus putting his/her finger into his/her mouth at 21 weeks of fetal age. Later, several times, both Misaki's and Mizuki's fetuses were seen putting their fingers or toes into their mouths. One hand also was seen several

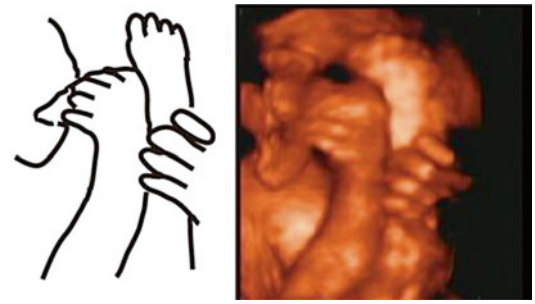

Fig. 4.6 Foot grasped by the hand by a chimpanzee fetus at 23 weeks of fetal age

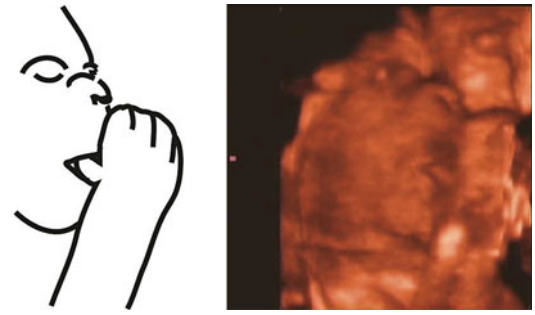

Fig. 4.7 Thumb into the mouth of a chimpanzee fetus at 23 weeks of fetal age

times holding the other hand or a foot (displayed in Fig. 4.6). Touching the hands and putting fingers in the mouth provide the fetus the experience of a double contact. When a foot is held with a hand, the holding hand feels the foot, and at the same time, the held foot feels the hand. When a finger is put in the mouth, the mouth is felt with the finger, and the finger is felt with the mouth. It is considered that, through these experiences, they recognize the integrity of their body, leading to an understanding of one's own body, as well as the ability to differentiate between self and the environment. Thus, it is possible that chimpanzees lay the foundation for an understanding of the self through the act of making contact with their own bodies during the fetal stage before birth.

Movements involving the mouth alone, such as opening or closing, became frequently visible after 20 weeks of fetal age. When Misaki's fetus was 22 weeks of fetal age, the ultrasound cross-sections of the fetus' face showed that it was moving rhythmically as if rippling the tongue. The movement was very similar to the suckling response during breastfeeding (shown in Fig. 4.7).

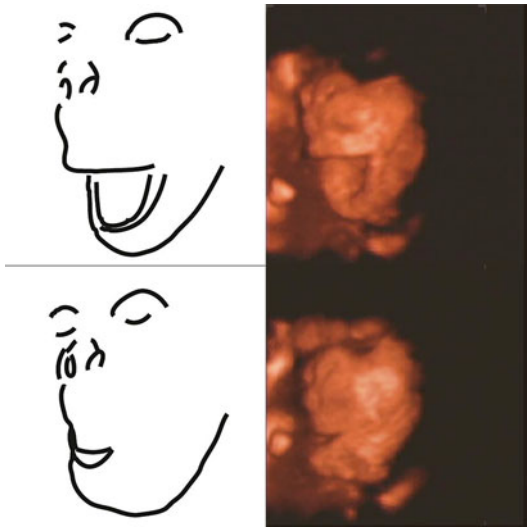

Fig. 4.8 Yawning by a chimpanzee fetus at 28 weeks of fetal age

The same action was confirmed in Mizuki's fetus for the first time at 26 weeks of fetal age. Thus, the suckling behavior that occurs immediately after birth has already been practiced in the womb and occurs even when they are not holding a nipple in the mouth. When Misaki's fetus was at 28 weeks of fetal age, he/she appeared to be yawning (see Fig. 4.8). He/she opened his/her mouth wide, then closed it slightly with the impression that the lips twisted slightly, and then again opened his/her mouth and soon closed it again. The movement was remarkably similar to a yawn. Yawning, too, is a behavior that can be seen in chimpanzee fetuses (Reissland, Francis, & Mason, 2012).

In humans, by 25 weeks of fetal age, the fetus has rotated, in the uterus and although sometimes he/she enters the breach position (i.e., with the buttocks or feet down), and by around 30 weeks of fetal age the head positions downward and settles into the mother's pelvis. In the three chimpanzee examples of Tsubaki, Mizuki, and Misaki, each of the fetuses had turned in the womb by 20–28 weeks of fetal age, assuming either the breach position or the head down position, but all three individuals settled into the head down position by 28 weeks of fetal age at the latest.

At 20–28 weeks of pregnancy in humans, women have remarkably large bellies, but pregnant chimpanzees do not have as noticeably as large of a belly as that in humans. In Tsubaki's case, there was less amniotic fluid compared to that in a human, and the womb was beginning to look tight. The fetal movement was restricted and it was difficult to observe it by using 4D-US. The same also was true for Misaki and Mizuki. They had less amniotic fluid than that in pregnant human women at this stage of pregnancy, and the fetuses are seemingly pushed into a relatively narrow space. Conversely, human fetuses develop in an environment that allows them relatively free body movements in the pregnant woman's uterus, which expands into the abdomen. From early to mid-development of the fetus, chimpanzees move their limbs in the same way as humans and perform similar physical exercises. However, from mid- to late development, they are in a narrower womb compared to humans, and limb movements are restricted. After birth, infant chimpanzees use their hands exclusively to cling to the mother. These prenatal differences in body movement might account for the species' differences in aspects of postnatal sensory-motor development. Human fetuses might have a higher degree of freedom of movement of the limbs because of a relatively larger intrauterine space and a larger amount of amniotic fluid. If differences in the quality and quantity of the movements of the limbs occur in such an environment, the impacts of the differences will affect the way in which the baby perceives him/herself, and no matter how primitive, this could lead to qualitative differences in self-perception.

The mean gestation period in chimpanzees is about 230 days. The neonate emerges from the mother's womb at around 32–33 weeks of fetal age. The neonate's body at this time weighs about 1800 g and the biparietal diameter is a little less than 7 cm. The human fetus at the corresponding fetal age weighs somewhat more, about 2000 g, and the head is slightly larger, with a biparietal diameter of 8.2 cm. The gestation period for humans is about 270 days. The human fetus continues to grow in the uterus for about 1 month longer than the chimpanzee fetus. Human neonates are born with a larger body and larger brain compared to the great apes. It seems that the

length of the gestational period and the speed of fetal development have been adjusted in the course of evolution for that reason.

Fetal Brain Growth in Humans and Chimpanzees

The differences in brain volume, and in other anatomical characteristics, may strongly relate to differences in behavioral expression between humans and nonhumans. It is known that the brain volume in newborn chimpanzees is about 150 cm^3, and that in humans it is about 400 cm^3. When and how these differences appear has been unknown, perhaps because of the paucity of research on the intrauterine development in nonhuman primates. Research on brain growth in chimpanzees has been largely limited to using postmortem fetuses, cranial samples, and/or using ultrasonography with anesthesia (Bourry, Ouwe-Missi-Oukem-Boyer, Blanchard, & Rouquet, 2006). Indeed a noninvasive means of observation to longitudinally study healthy developing fetuses is preferable. Such research is now possible owing to the availability of pregnant chimpanzees who have built close relationships with researchers who have raised them. Images were taken of the fetuses of two pregnant chimpanzees (Mizuki & Misaki) from 9 weeks of gestation to shortly before birth, at the Great Ape Research Institute, Hayashibara Biochemical Laboratories (Sakai et al., 2012). The study showed that the brain volume of the fetuses at 16 weeks of gestation were half the brain volume of a human fetus at the same time (see Fig. 4.9, panel a). Shortly after this, the fetuses' brain volume growth velocity began to accelerate. At 16 weeks, the brain volume was observed to be growing at an estimated velocity of 6 cm^3/week. One week later, there was an increase of 6 cm^3 in the brain volume, and at around 22 weeks, it was growing at a rate of more than 10 cm^3/week. For humans as well, brain volume growth accelerates and growth velocity increases. Until about 22 weeks of gestation, this pattern of acceleration in the brain volume growth was the same in chimpanzees and humans. However, while the human brain volume continued to increase rapidly until about 32 weeks of gestation, for the chimpanzee fetuses, a different growth pattern appeared (see Fig. 4.9, panel b). The chimpanzee fetuses' brain volume showed the same velocity of development as in human fetuses from 17 weeks to around 22 weeks, but at around 22 weeks, the acceleration of development leveled off. At 32 weeks, compared to the 26.1 cm^3/week growth velocity in the human fetuses, the brain-volume growth velocity of the chimpanzee fetuses was only 4.1 cm^3/week. Based upon these results, it would appear that chimpanzee brain volume does not much increase after the second trimester. Thus, growth acceleration stopped in the chimpanzees in the second trimester in contrast to humans.

In humans, brain-volume growth velocity is assumed to accelerate until shortly before birth (Fujimura & Seryu, 1977; Roelfsema, Hop, Boito, & Wladimiroff, 2004). In contrast, in chimpanzees the acceleration stops in the second trimester of pregnancy. In other words, this specific and extraordinary brain enlargement in humans begins at the fetal stage. The pattern of development in which there is a continuous acceleration in brain volume growth velocity through the late fetal stage may be thought of as a characteristic uniquely acquired in the lineage of humans after divergence from the lineage of common ancestor of humans and chimpanzees.

However, brain enlargement is not the only feature in humans. Studies of the percentage of brain weight in relation to body weight in newborns have shown that it is about 12.3 % in humans, which is 12 % in almost all primates (DeSilva, 2011). Indeed, there are exceptions in the great apes, where the brain weight is about 10.0–10.1 %. Thus, among currently existing primates, it seems that only the offspring of the great apes are born with small brains relative to their body weight. It may be noted that among primates, humans are not born with a conspicuously "big head." Relative to body size, the head of humans is larger than that of the great apes, but this relationship is about the same as in other primates, or slightly larger.

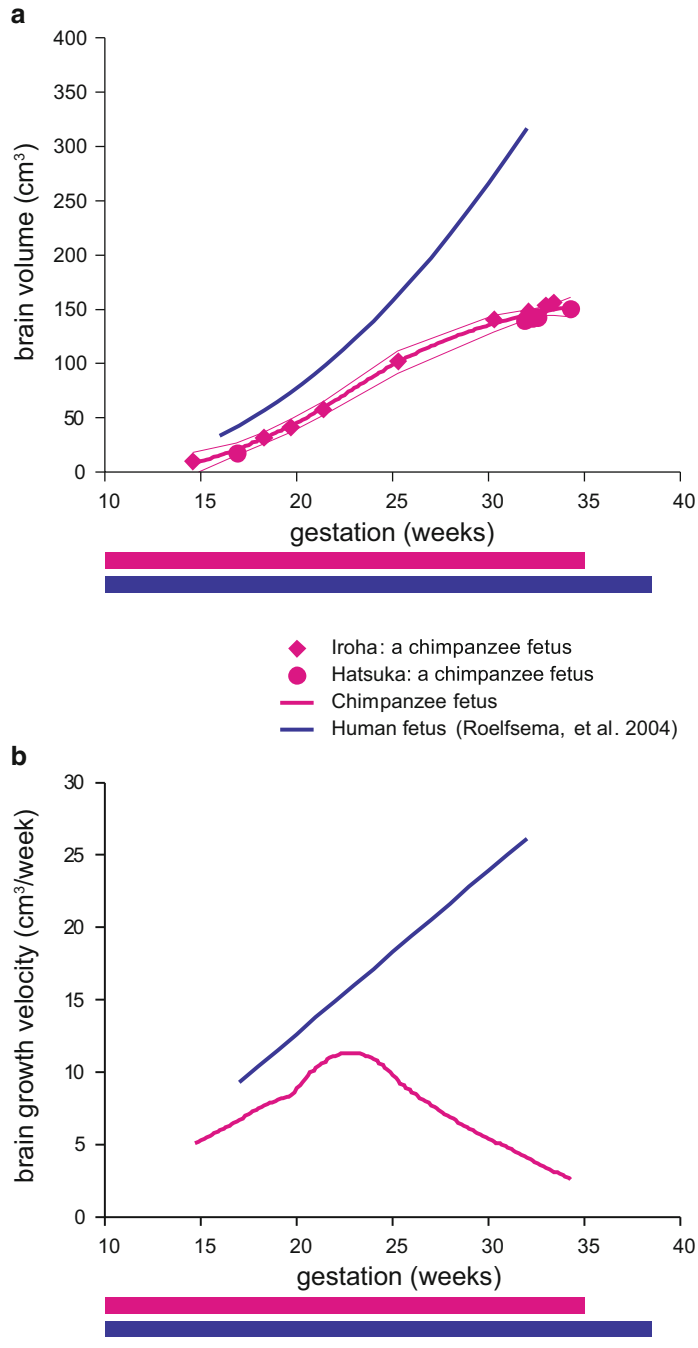

Fig. 4.9 (**a**) Gestational age-related changes in the brain volume and (**b**) in the growth velocity of brain volume

Brain Growth Delay/Deceleration and the Development of Postural Reactions

Regardless of the primacy of brain size or body size, both grow in tandem in primates (Deacon, 1990). However, it is possible that species differ in aspects of growth velocity and the timing of its deceleration. For example, the fact that the percentage of brain weight relative to the body weight of newborns is 12 % for other species of primates and 10 % for the great apes suggests that the

Table 4.1 Comparison of the timing in the fetal period at which brain growth acceleration ceases or decelerates and the timing after birth at which the transition to the developmental stage for postural reactions takes place among baboons, chimpanzees, and humans

				(Days after conception)	
				Human	
		Baboon	Chimpanzee	Fullterm	Preterm
A	Cessation or deceleration of brain growth acceleration	130	160	210	260
B	Birth	190	230	270	220
C	Onset of the 2nd stage of postural reactions	200	310	410	410
D	Onset of the 3rd stage of postural reactions	220	350	470	470
E	Onset of the 4th stage of postural reactions	280	500	630	630
$1-A/B$		0.32	0.30	0.22	−0.18
$1-A/C$		0.35	0.48	0.49	0.37
$1-A/D$		0.41	0.54	0.55	0.45
$1-A/E$		0.54	0.68	0.67	0.59

development of uniquely large bodies in the great apes may begin in the fetal stage. This would suggest that in the human fetal stage, extraordinary brain enlargement evolves (with accelerating velocity) in addition to body enlargement shared with the great apes (i.e., it is accompanied by a species-specific evolution in body enlargement). If through future research we can better understand prenatal brain and body development in the great apes and other primates, we may come one step closer to solving the mystery of extraordinary body enlargement in the hominoids and the developmental evolution of extraordinary brain size in humans.

With reference to motherhood, a mother's labor related to pregnancy and birth becomes more difficult when children have larger brains and bodies. During human evolution, it would have been advantageous to choose the best time to give birth in order to minimize problems in delivery and caring for the child. How might the status and growth of the child in the uterus and the progress of the mother's pregnancy affect the timing of birth? According to Portmann's theory of physiological prematurity (Portmann, 1951), human infants are born a year early compared to the birth of other mammals. Based on findings in comparative behavioral developmental research, such a statement may be difficult to justify. However, by focusing on the transition period for the development of postural reactions, and considering this period to be a few months, rather than 1 year, there may be room for discussion.

Table 4.1 compares the timing in the fetal period (number of days after conception) at which brain growth acceleration ceases or decelerates and the timing after birth (number of days after conception) at which the transition to the developmental stage for postural reactions (Takeshita, Tanaka, & Matsuzawa, 1989, Fig. 4.10) takes place. When we obtain values from "the length by the time of gestation at which brain growth acceleration ceases or decelerates (*A*, Table 4.1)" and "the length of the gestation period (*B*, Table 4.1)" for baboons (Liu et al., 2009) and for chimpanzees (Sakai et al., 2012) through a formula of $1-A/B$ as a relative timing of "brain growth acceleration ceases or decelerates," both would be almost the same (for baboons 0.32 and for chimpanzees 0.30, see Table 4.1). Could it be tentatively concluded that the timing of birth in relation to the cessation or deceleration of brain volume growth acceleration is about the same for both baboons and chimpanzees. If so, what might that say about the continuation of acceleration of brain growth for a few months before and after birth in humans?

There has been almost nothing written in the literature regarding the brain volume growth velocity in human fetuses up until shortly before birth. We assumed that the occipitofrontal head circumference correlates closely with brain size,

Fig. 4.10 Development of postural reactions in primate infants. Numbers in the photos represent each participant's age in weeks

and used the data from Fujimura and Seryu (1977) for Table 4.1. According to Fujimura and Seryu, the occipitofrontal head circumference of the human fetus reaches its peak at 31 weeks of gestation. However, if preterm birth takes place at 30–33 weeks, the deceleration is delayed, and the peak is not reached until around 37 weeks after conception, when deceleration subsequently begins. Thus, when the human fetus reaches a certain size in the narrow uterus, growth is suppressed, but as the fetus is released from the uterus in a preterm birth, the accelerated growth resumes. Using Fujimura and Seryu's groupings, the fullterm infants (the former case) comprised the Human Fullterm, and the preterm infants (the latter case) comprised the Human Preterm. The results have been presented in Table 4.1. First, regarding the cessation or deceleration of brain growth acceleration (A) and its relationship to the length of the fetal period (B), Human Fullterm (0.22 from $1-A/B$) exhibited the "baboon = chimpanzee pattern (0.30–0.32)" with a small modification, and Human Preterm (−0.18) exhibited a large modification. Next, for each group, looking at the gestation time for reaching the second, third, and fourth stages of the development of postural reactions (C, D, E, respectively) in relation to the timing of cessation or deceleration of brain growth acceleration, as compared to the baboons (0.35, 0.41, and 0.54 from $1-A/C$, $1-A/D$, and $1-A/E$, respectively), the chimpanzees (0.48, 0.54, and 0.68) needed a longer time for the transition to each stage, which may indicate a developmental delay for postural reactions in chimpanzees. In addition, the data showed that the Human Fullterm (0.49, 0.55, and 0.67) followed the chimpanzee pattern, and Human Preterm (0.37, 0.45, and 0.59) followed the baboon pattern (because duration of the fetal period of baboons is almost equal to that of the macaques, the postural reaction stage data used here followed the data for the macaques). Human Fullterm, which had fullterm births of 270 days of gestation, closely followed the chimpanzee pattern of postural reaction development after birth. In other words, when there was a relationship between the stage of postural reaction development and brain growth velocity, a delay in human development is not indicated, because the timing of birth is just a little earlier than what would be expected. Does this indicate that if there is sufficient room in the uterus for the fetus to stay, postural reactions after birth may follow the baboon pattern of development velocity? Although there might be cases where the development of postural reactions in preterm infants differs from the schedule for the fullterm infants, here we would like to bring attention to the apparent adjustment of birth timing to allow the maximum possible time for development in the uterus, and that the humans typically closely follow the chimpanzee pattern of development.

Time of Birth and the Ways Children Grow and Are Raised

We have looked at the possibility of a relationship between the time of birth and the ceasing or deceleration of brain growth acceleration in primate species. However, there also may be a relationship to an adaptive development of the ability of the forelimbs to support the body. It seems that in almost all primates, birth may take place when the baby is able to support his/her body up against gravity by extending its forelimbs. Compared to the offspring of many mammals that follow their mothers under their own power as newborns, one could say that there is a developmental delay in posture and locomotion in primates. In addition, a further delay occurs in humans. Even fullterm newborns are helplessly dependent on their mother. The need to carry the human infant around day and night continues for a relatively long time, as compared to other primates. During that time, the mother accepts the clinging child and nurtures, as do the great apes. In contrast with great apes, it is said that humans are physically premature at birth. The accelerated growth of the brain and body continues without pause, therefore they must be born "early," and the newborn is conspicuously immature and powerless compared with the babies of great apes. However, a human mother has the ability to carefully raise the newborn. She would have acquired the ability to walk upright on two feet, to hold the baby in her arms and, in paying

attention to the helpless child, would have improved her ability to care for it. In addition, there would have been various opportunities for the adults surrounding the mother to support child rearing. The mother would probably also have communicated with the child and built social relationships with others who helped her in child rearing (Hrdy, 2009).

Acceleration in brain growth during the fetal period is found in the genetically closest chimpanzee, but in humans, it is more rapid and continues for longer. The reality behind the acceleration of the brain growth in human fetuses, beginning at 22 weeks of gestation, is assumed to lie in various phenomena related to the formation of neural circuits, such as the development of axons and subplate cells, synaptic connections, and glial cell proliferation (e.g., Linderkamp, Janus, Linder, & Skoruppa, 2009). Quantitative and qualitative growth of this kind of brain tissue, which happens after cell migration is completed, beginning in the second trimester of pregnancy, could be the initial developmental period for the wide range of human behaviors related to our "degrees of freedom" (e.g., Thelen & Smith, 1996), like the ability to manipulate fingers, recognize oneself and others, and, furthermore, interact with others in sophisticated ways (e.g., multinational business transactions).

Using 4D-US to observe behavioral indicators such as facial expression in addition to heartbeat, we have observed aspects of fetal communication with the outside world. In humans, studies using heart rate as an indicator have shown that even before birth, fetuses are capable of distinguishing different acoustic stimuli, such as the voice of their mother from that of other women (Kisilevsky et al., 2003). In addition, findings from studies conducted by us, in which mouth movements were used as an indicator, have suggested the possibility that such an ability might have developed at an earlier stage. Human fetuses opened their mouths more frequently when they heard the voice of their mother than when they heard the voice of other women. In humans, it is apparent that a fetus shows a unique sensitivity to the voice of his/her mother. In itself, the fact that the sound of the mother's voice makes the fetus open his/her mouth more frequently is interesting in relation to the early development of the understanding of the "self" and others (Myowa-Yamakoshi et al., unpublished data). Assuming that this kind of communication exists between human mothers and their children, does the fetus already have dual coding of experience and emotions at this stage? How about mother and baby chimpanzees?

When, why, and how did human development take this singular path seen here? The source must have been the intertwining changes that related the ways children grew and were brought up both before and after birth. Those changes are the driving force for human evolution, and for the creation of humanness. We hope to further expand our research into related areas of human development from this perspective.

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Part II

Brain-Behavior Development: Human Fetuses

Fetal Behavior: Clinical and Experimental Research in the Human

5

Eduard J.H. Mulder and Gerard H.A. Visser

Abstract

The intrauterine environment in which we develop has an enormous impact on our physiology and behavior, displayed not only before but also after birth. Proper knowledge of normal fetal behavior, reflecting central nervous system functioning, is essential to detect and characterize the effect on fetal behavioral development of a particular antenatal disturbance. The latter may relate to fetal structural and/or genetic abnormalities, to fetal exposure to poor nutrition or recreational and (non-) prescription drugs, and to maternal physical and mental diseases. This chapter summarizes our experience with neurobehavioral development in the human fetus, studied in clinical and experimental conditions by two-dimensional ultrasonography and spanning several decades.

Keywords

Fetal behavior • Fetal motility • Fetal movement patterns • Fetal heart rate • Fetal behavioral states • Fetal sleep states • Normal pregnancy • Complicated pregnancy • Ultrasound • Ultrasonography

Introduction

"Is our baby all right?" is one of the first questions new parents are likely to ask after delivery. The universal fear of a malformed or functionally disabled infant is fully understandable as pregnancy does not always end in the birth of a normal healthy infant.

Several test procedures are presently available for screening and diagnosis of fetal genetic abnormalities and structural malformations. However,

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prenatal diagnosis of functional anomalies related to the developing central nervous system (CNS) remains difficult. Fetal neurological disorders are sometimes present in the absence of structural deficits and may not be recognizable until after birth. They also may emerge during postnatal development and do not become apparent until later in life. There is increasing awareness that many of these neurological disorders, including ADHD and autism, may be induced during the prenatal period (Van den Bergh, 2011). Variations in the fetal environment have been shown to exert significant long-term or even permanent effects on behavioral, physiological, and cognitive development in offspring (Gluckman & Hanson, 2006). Environmental influences that bear potentially adverse risks for fetal development relate to maternal factors, such as disease, psychological state, and complications during pregnancy, and to exposure to exogenous substances (pollutants, recreational and medicinal drugs).

Although there is no universally accepted procedure to test CNS-functioning in the fetus to date, much has been achieved in fetal neurology over the last 40 years. The introduction of dynamic imaging ultrasound made it possible to observe the fetus safely, directly, and repeatedly in its natural environment allowing the accurate assessment of spontaneous human fetal behavior. The year 1982 represents a research milestone in this respect, as three important articles appeared that dealt with (a) the emergence of embryonic/fetal movement patterns during the first trimester (de Vries, Visser, & Prechtl, 1982); (b) the emergence of fetal behavioral states in the third trimester (Nijhuis, Prechtl, Martin, & Bots, 1982); and (c) the concept and methodology of behavioral states in the newborn (Prechtl & O'Brien, 1982). These articles paved the way to subsequent studies of fetal behavior in normal and disturbed situations (Visser & Mulder, 2009). It was clear from the beginning that no investigation of abnormal behavioral development proceeds far without the recognition that there are large variations in "normality." Hence, obvious sources of inter- and intra-fetal variation [e.g., ultradian (quiet and active sleep states) and diurnal rhythms] have to be carefully controlled for.

Fetal movements are spontaneous expressions of the CNS and fetal sleep regulation is a well-controlled complex behavior reflecting the primary brain activity at the end of pregnancy. Measures of the normal occurrence and development of fetal motor patterns and behavioral states are therefore candidates for diagnostic purposes (i.e., for assessing fetal CNS integrity). This knowledge is essential to differentiate between the fetus that is healthy and the one that is (acutely) distressed, deteriorating, or has a chronic dysfunction. The ultimate goal of the study of fetal behavior is to detect the effects of a particular antenatal disturbance and also to characterize these effects.

In this chapter, we report results obtained by members of our research team with the use of real-time two-dimensional (2D) ultrasonography. Newer technologies are presently available for fetal neurobehavioral assessment: 4D ultrasonography (see Chaps. 10 and 9, by Kadic et al.; and Reissland, Francis, & Buttanshaw); HDlive (see Chap. 19 by Hata et al.); and biomagnetometry (see Chap. 23 by Gustafson & Popescu).

Normal Development of Fetal Behavior

Systematic 2D ultrasound observation has provided ample knowledge of the ontogeny of spontaneous behavior in the human embryo and fetus (de Vries & Fong, 2006; Felt et al., 2012; Visser, Mulder, & Prechtl, 1992; see Chap. 6 by Nijhuis). In short, embryonic movements ("sideways bending") can be first detected at 7 weeks postmenstrual age (5 weeks after conception). They are followed by the emergence of a set of specific movement patterns, such that the fetus exhibits an impressive repertoire by 15 weeks of pregnancy, including generalized body movements (GM), breathing, and head, limb, mouth, and eye movements (as displayed in Figs. 5.1 and 5.2). For each pattern, the developmental trajectory has been studied in terms of age at emergence, range of occurrence, and change over time and in relation to other movement types. For instance, GM occur randomly during the first half of gestation. They

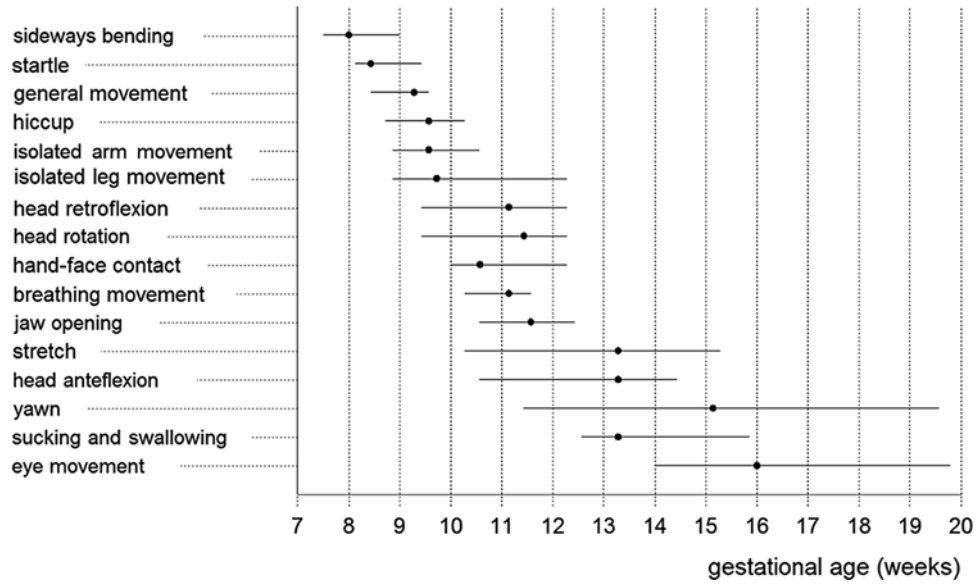

Fig. 5.1 Emergence of specific fetal movement patterns with time presented as median and range of first appearance during serial observations on individuals. Reproduced from de Vries et al. (1982), with permission

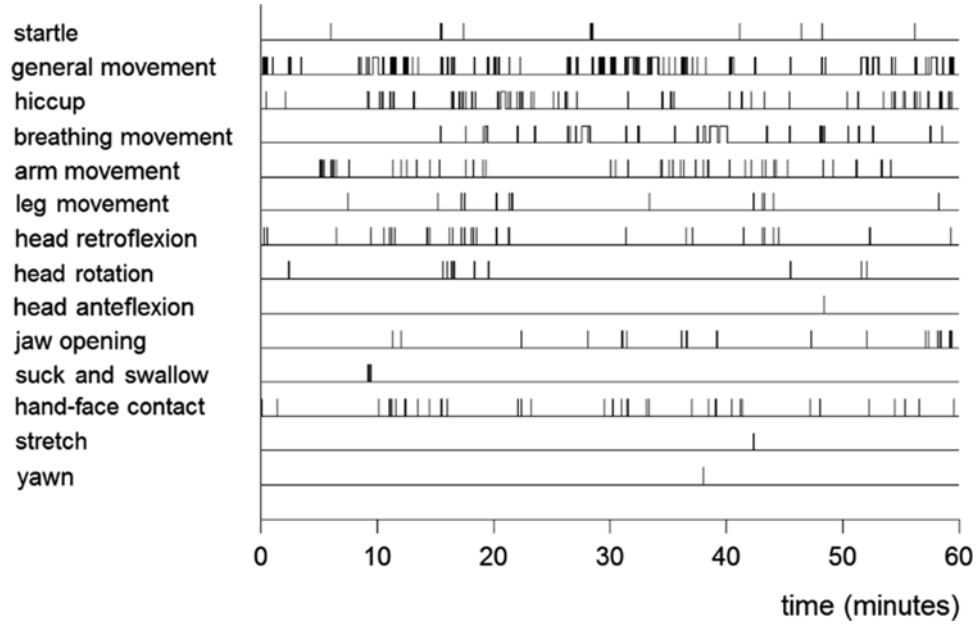

Fig. 5.2 Compiled actogram of a fetus at 15 weeks of gestation during 1 h of observation. Reproduced from de Vries et al. (1982), with permission

become progressively clustered to appear as phases of rest and activity around 28 weeks of gestation, which are fairly associated with patterns of low and high heart rate variability, respectively (Nijhuis et al., 1998a; ten Hof et al., 1999). During the third trimester, episodes of rest and activity and their heart rate patterns become increasingly linked to absence and presence of rapid eye movements (REM), respectively (Hof et al., 2002; Nijhuis et al., 1998b; Visser, Mulder,

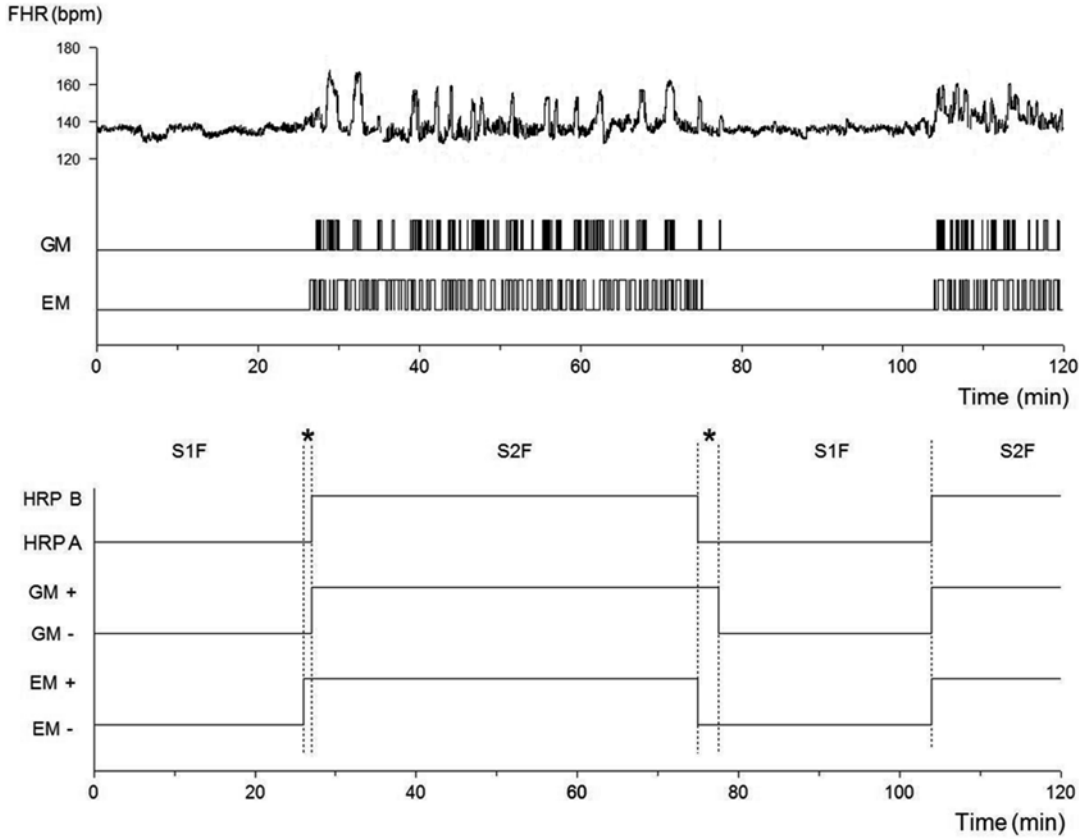

Fig. 5.3 Example of a 2 h fetal behavioral state recording made at 38 weeks of gestation from a healthy pregnant woman. Presented are the original data for fetal heart rate (FHR), general movements (GMs), and rapid eye movements (REMs; *upper panel*); and the smoothed state profile indicating episodes of FHR pattern (HRPs A and B), and the presence (+) and absence (−) of GMs and EMs (*lower panel*). S1F, state 1F; S2F, state 2F; *no-state identified/transition. Reproduced from Mulder, Ververs, de Heus, and Visser (2011), with permission

Stevens, & Verweij, 1993). Eventually, stable non-REM (quiet) and REM (active) sleep states with sharp transitions are present near term (Nijhuis et al., 1999) as shown in Fig. 5.3. We found no differences in the development of fetal movements, heart rate (patterns), or states between boys and girls (Robles de Medina, Visser, Huizink, Buitelaar, & Mulder, 2003).

The study of fetal behavior has involved both quantitative and qualitative analysis of movements (de Vries & Fong, 2006). With the former, the number of events and duration of activity bursts are determined and expressed as % incidence; this can be done for all movement patterns. In the presence of fetal states the occurrence of some movement patterns (GM, breathing) may be provided as % of time for each state separately. Quality assessment is confined to fetal general movements, as GM comprise a complex variable pattern, involving movements of the body, limbs, and head in a random order (Prechtl, 1990). Normal quality is characterized by variability in speed, amplitude, complexity, and fluency of the movement (i.e., non-stereotypy). On occasions, fetal breathing movements are qualitatively described as being vigorous, of large amplitude, or shallow.

Quantitative and qualitative features of normal behavioral development have proven to be a powerful means of detecting variations in fetal CNS functioning at different stages of antenatal life and under normal and abnormal conditions. Examples of altered neurobehavioral development, mainly from our research center, are reviewed in the remainder of this chapter. See Table 5.1 for a list of the conditions examined.

Table 5.1 Effect of some pregnancy complications and environmental factors on the quantity and quality of fetal general movements (GM) and on behavioral state organization

	GM quantity	GM quality	Sleep states
Congenital malformations			
Hypokinesia type	X	X	
Hyperkinesia type	X	X	
IUGR	X	X	X
Maternal diabetes			X
Preterm labor/PPROM		X	
Alcohol			X
Caffeine	X		X
Corticosteroids	X		X
Antihypertensive/tocolytic drugs			
Antidepressants (SSRI)	X		X
Cocaine	X		X
Antiepileptic drugs			
Maternal stress	X		X
Vibro-acoustic stimulation	X	X	X

X: affected

Clinical Conditions Affecting Fetal Behavior

Clinicians are most interested in the diagnostic value of behavioral measures as these may shed light on fetal well-being or developmental deterioration. Several aberrations of the normal developmental course have been reported under varying clinical conditions. These abnormalities involve delayed emergence of movements, changes in the quantity (extremely low or high occurrence rate) or the quality of motor performance, and abnormal development of fetal behavioral states.

Altered Fetal CNS, Skeletal, or Neuromuscular Development

Alterations in fetal behavioral development have been found to be associated with a variety of structural or chromosomal abnormalities. Based on a series of affected cases, distinction has been made between hypokinetic and hyperkinetic motility patterns (de Vries & Fong, 2007).

Hypokinesia is often found among fetuses with a neuromuscular, skeletal, or skin disorder of a genetically autosomal recessive etiology (de Vries & Fong, 2007). The quality of their GM is characterized by a reduction in amplitude, speed, and number of participating body parts. GM quantity is absent or highly reduced. During prolonged fetal heart rate monitoring, episodes of (extremely) low variability for more than 45 min may be indicative of a hypokinetic fetus. In a few case reports involving repeat scans on individual fetuses, evidence was found that the normal reciprocal structure–function relationship deteriorates with time (Mulder, Beemer, & Stoutenbeek, 2001; Mulder, Nikkels, & Visser, 2001). This phenomenon is known as the fetal akinesia deformation sequence (FADS). It is presumed that absence or low incidence of fetal body and limb movements, breathing activity, and mouth movements (sucking and swallowing) underlie the occurrence of joint contractures, pulmonary hypoplasia, and facial anomalies and polyhydramnios, respectively.

Hyperkinesia occurs in fetuses affected by rare structural or genetic disorders that involve the CNS (de Vries & Fong, 2007). Assessment of GM quality shows increased amplitude and speed, and seizure-like phenomena on occasions. GM quantity may range from a normal rate of occurrence to almost continuous activity. The latter, characterized as forceful chaotic moving, was seen in anencephalic fetuses with extended brain defects (Visser, Laurini, de Vries, Bekedam, & Prechtl, 1985). As GM are normally generated at the brainstem and spinal levels and are modulated by higher centers, these case observations demonstrate that GM quantity and quality decay if higher brain structures are impaired.

However, prenatal prediction of neurologic outcome based on altered fetal behavior remains difficult. It requires not only extensive knowledge of normal movements but also multiple diagnostic approaches.

Intrauterine Growth Restriction (IUGR)

Fetal growth restriction may result from diminished nutrient and oxygen supply to the fetus in pregnancies complicated by uteroplacental

insufficiency. During deterioration, there appears to be a sequence of changes with time in fetal physiological and behavioral variables, known as the adaptation/decompensation cascade of fetal growth restriction (Ribbert, Visser, Mulder, Zonneveld, & Morssink, 1993; Rizzo, Arduini, Pennestri, Romanini, & Mancuso, 1987; Turan, Miller, & Baschat, 2008). Initially, the quantities of GM and breathing movements fall within the normal range, but they decrease in incidence at or shortly after the onset of fetal hypoxemia. This is followed by a further decline in movement incidence and a rapid fall in heart rate variability when deterioration progresses to acidemia and poor fetal condition (Bekedam, Visser, Mulder, & Poelmann-Weesjes, 1987; Nijhuis et al., 2000; Snijders, Ribbert, Visser, & Mulder, 1992). Quantitative changes in movements are thus relative late signs of impairment and are preceded by abnormalities in blood flow and heart rate parameters and altered GM quality. Growth-restricted fetuses show less complex GM and do so also after birth (Bekedam, Visser, de Vries, & Prechtl, 1985; Sival, Visser, & Prechtl, 1992). Their GM are slow, monotonous and of small amplitude, reflecting the CNS condition in utero which is likely hampered by chronic malnutrition rather than hypoxemia. The development of behavioral states is delayed and/or disturbed in growth-restricted fetuses, resulting in abnormal state organization near-term (Arduini et al., 1989; van Vliet, Martin, Nijhuis, & Prechtl, 1985). No-coincidence of state parameters (functional chaos) was high and fetuses spent more time in quiet sleep (state 1F) than in active sleep (state 2F). In this way, IUGR fetuses may reduce the cost of energy expenditure, which suggests fetal adaptation to the poor intrauterine milieu.

Maternal Diabetes

Embryonic/fetal growth and behavioral development have been studied longitudinally in pregnant women with type-1 diabetes (Mulder, 1993). Compared with first trimester controls, embryos and fetuses were often smaller (early growth delay), but showed the same set of specific movement patterns and similar developmental trajectories for each pattern. However, the emergence of all but one of the movement patterns was delayed by 1–2 weeks. The exception was fetal breathing. This activity emerged earlier in the embryos of diabetic women, reached higher incidence values throughout pregnancy, was performed vigorously, but occurred at a lower breathing rate (Mulder, Leiblum, & Visser, 1995; Mulder & Visser, 1991a, 1991b; Mulder, Visser, Morssink, & de Vries, 1991).

During the third trimester, the emergence of behavioral states was disturbed in fetuses of diabetic women (Mulder, Visser, Bekedam, & Prechtl, 1987). This resulted in poorly organized states at the end of pregnancy and at 1 week after birth (Mulder, O'Brien, Lems, Visser, & Prechtl, 1990). Disturbances found included a high proportion of no-coincidence and lack of achieving proper synchrony of parameter changes at transitions. No state variable in particular (GM, EM, or heart rate pattern) was found to be the principal cause of poor association. Macrosomic fetuses were characterized by a low occurrence of quiet sleep (state 1F) and prolonged activity (GM) cycles. Poor state control was especially found in the fetuses with a history of early growth delay (Mulder & Visser, 1992). This stresses the impact of early disturbance on subsequent functional development, and supports the relationship between early growth delay and impaired psychomotor performance in 4-year-old infants of diabetic mothers reported by others (Bloch Petersen, Pedersen, Greisen, Pedersen, & Mölsted-Pedersen, 1988). Diabetes in pregnancy has profound effects on fetal growth and behavior. The importance of degree and timing of maternal glucose control is still a matter of controversy (Mulder, Koopman, Vermunt, de Valk, & Visser, 2010). Although infants of diabetic women generally develop favorably in later life, some, especially when born macrosomic, continue to run an increased risk of developmental disorders (Mulder, 1993; Rijpert et al., 2011).

Preterm Labor and/or Ruptured Fetal Membranes

In women admitted for preterm contractions with or without ruptured membranes, the quantities of fetal GM and breathing movements are usually within the normal range showing wide inter- and intra-fetal variation (Devoe, Youssef, Croom, & Watson, 1994). These movements are considered unreliable as an indicator of an individual fetus' neurologic condition. In case of preterm rupture of the membranes, the quality of GM changed with increasing reduction of the amount of amniotic fluid (oligohydramnios) (Sival, Visser, & Prechtl, 1990). GM amplitude decreased first, followed by a decrease in speed during the process of increasing mechanical constraint. GM remained forceful. Postnatally, the small amplitude and low speed of GM tended to normalize between 1 and 5 weeks.

Fetal breathing movements are arrested during labor, likely due to increased prostaglandin levels (Harding, 1997). Hence, presence of breathing might be a good marker of low risk for delivery in women with preterm contractions (Besinger, Compton, & Hayaski, 1987; Devoe et al., 1994). Although initial studies on the subject were promising, the association did not hold when evaluated in a systematic review (Honest et al., 2004).

Twin Pregnancy

Behavioral studies on fetal twins are few in number. They are marked by technical, methodological, and conceptual differences and have been recently reviewed (Tendais, Visser, Figueiredo, Montenegro, & Mulder, 2013). Several studies have reported on a high occurrence of simultaneous fetal movements and extensive intra-pair contacts between twin members, as well as on inter-twin simultaneity of rest–activity cycles or sleep states. However, this view has been challenged in a study of behavioral development in normal dichorionic twins demonstrating a considerable chance effect (Mulder, Derks, de Laat, & Visser, 2012).

Recreational, Nonprescription, and Prescription Drugs; Behavioral Teratology

At present, most patients and physicians are well aware that the use of recreational drugs (e.g., alcohol, coffee, tobacco), nonprescription drugs (e.g., cocaine), and prescription (medicinal) drugs during pregnancy might have adverse effects on the embryo and fetus. Recreational and nonprescription drugs should not be taken when pregnant, and prescription and consumption of medication should be minimized if therapeutic benefits outweigh the possible risks. However, the ideal of drug-avoidance cannot always be fulfilled: fetal exposure may occur because the woman is unaware that she is pregnant, or because there is lack of knowledge that the drug might adversely affect the unborn. Moreover, in the case of some diseases, such as diabetes mellitus, hypothyroidism, hypertension and epilepsy, drug therapy during pregnancy is unavoidable without endangering the lives of the mother and fetus.

The discipline of fetal behavioral teratology deals with the recognition, detection and prevention of the causes of prenatally induced neurodevelopmental disorders that may be permanent and irreversible (Werboff & Gottlieb, 1963). So far, solid evidence that a particular substance is a behavioral teratogen is confined to only a few substances, including alcohol, methyl mercury and lead, while some are suspect (phenobarbital, opiates, DES). Information on substances under suspicion of behavioral teratogenicity usually comes from human epidemiological and case studies (Källén, Borg, & Reis, 2013). However, this information is obtained during the postnatal period, where interactions with the (social) environment may have interfered with the in utero effects of drug exposure. Therefore, the search for human behavioral teratogens should begin far before birth. Ultrasound observations of fetal behavior may be helpful to provide direct evidence.

In the following, we present examples of fetal effects of drugs that are temporarily or chronically utilized during pregnancy. This is not to say

that we consider these as (potential) behavioral teratogens; they are meant to illustrate observable drug effects in the fetus.

Recreational Drugs

Alcohol

High alcohol consumption during pregnancy can cause the fetal alcohol syndrome (FAS), characterized by affected growth, CNS damage, abnormal craniofacial features (including eye malformations), and behavioral and cognitive disabilities in later life (Huizink & Mulder, 2006; see Chap. 17 by Dobson et al.). To see what happens during exposure to alcohol, we studied fetal behavior while the mother drank two glasses of white wine. The study, performed at term age, was carefully controlled for fetal sleep states (Mulder, Morssink, Benschop, & Visser, 1998). Fetal state cycling was disturbed during maternal drinking, caused by suppression of rapid eye movements, and breathing activity was drastically reduced. REM sleep is important for normal brain development (Darnall, Ariagno, & Kinney, 2006). Although speculative, recurrent alcohol-induced disturbance of fetal REM-sleep may underlie some neurobehavioral deficits seen with FAS. In a similar vein, ophthalmic abnormalities often seen in FAS infants may relate to fetal eyeball immobility each time the mother drinks (disturbed structure–function relationship) (Cook, Sulik, & Wright, 2003; Green, Munoz, Nikkel, & Reynolds, 2007). Knowledge of these direct effects of alcohol on the fetus may discourage pregnant women from drinking.

Caffeine

Caffeine is a CNS stimulant, also in the fetus. This was shown in a complex study design involving fetal ultrasound observation under normal maternal lifestyle conditions and again following intake of two cups of coffee, intermitted by abstinence of caffeine-containing products for 2 days (Mulder, Tegaldo, Bruschettini, & Visser, 2010). Under normal conditions, the %GM was positively related to maternal blood caffeine levels. However, fetuses not used to caffeine spent more time in wakefulness (State 4F) after maternal coffee loading, while fetuses of habitual consumers remained unaffected (i.e., they demonstrated normal cycling of sleep states only). The latter finding suggests tolerance to caffeine, probably due to programming of adenosine receptors in the regularly caffeine-exposed fetus (Fredholm, Chen, Masino, & Vaugeois, 2005). Fetal breathing was not influenced in this study. This opposes the clinical effect of caffeine to stimulate respiration in preterm born infants. It is presently unknown if tolerance to caffeine (temporarily) hampers successful anti-apnea therapy in these infants. Long-term neurodevelopmental outcome is generally favorable both in cases of high antenatal caffeine exposure and in infants treated for apnea of prematurity (Schoen, Yu, Stockmann, Spigarelli, & Sherwin, 2014).

Nonprescription Drugs

Cocaine

Disorganized fetal and neonatal behavioral states have been described after in utero exposure to cocaine, characterized by more wakefulness and arousal at the cost of quiet sleep (Hume, O'Donnell, Stanger, Killam, & Gingras, 1989).

Prescription Drugs

Synthetic Corticosteroids

Betamethasone and dexamethasone are routinely administered to women at risk of preterm delivery before 34 weeks of gestation. These potent drugs have beneficial effects on fetal organ maturation, the lungs in particular, but also have important side-effects (Mulder, Derks, Zonneveld, Bruinse, & Visser, 1994). The short-term (transient) effects of the corticosteroids comprise considerable reductions in the incidences of fetal GM and breathing and in heart rate variability 1–3 days after the start of therapy (Derks, Mulder, & Visser, 1995; Lunshof et al., 2005). GM quality is unaffected during therapy. Betamethasone affects the ultradian rest–activity cycle resulting in prolonged periods of fetal quiescence associated with (very)

low FHR variation, which are not normal for the age at occurrence (Mulder, Koenen, Blom, & Visser, 2004). These effects have been found to occur similarly in singleton and twin fetuses (Mulder, Derks, & Visser, 2004). Betamethasone also suppresses the normally occurring diurnal rhythms of fetal movements and heart rate (de Heus, Mulder, Derks, Koenen, & Visser, 2008; Koenen, Mulder, Wijnberger, & Visser, 2005). Dexamethasone compared with betamethasone has less dramatic effects on fetal behavior and heart rate, but its therapeutic effects also may be less favorable (Mulder, Derks, & Visser, 1997). Importantly, the corticosteroid-induced effects are not caused by fetal hypoxemia, as evidenced by absent FHR decelerations, but likely result from glucocorticoid receptor-mediated processes in the fetal brain (Cohlen, Stigter, Derks, Mulder, & Visser, 1996). Knowledge of the corticosteroid-related physiological effects on the fetal cardiovascular system, HPA-axis, and brain is helpful for clinical decision-making to minimize the risk of unwarranted iatrogenic preterm delivery (Mulder, de Heus, & Visser, 2009). There is presently no indication that one course of prenatally administrated corticosteroids affects long-term developmental outcomes, but this may not hold for repeat antenatal courses (Crowther & Harding, 2007).

Antihypertensive and Tocolytic Drugs

Altered behavioral state distribution has been found in fetuses of women treated with antihypertensive drugs (labetalol, nifedipine) for pregnancy-induced hypertension (Gazzolo et al., 1998). The fetuses of treated women compared with control fetuses showed more no-coincidence and State 1F, at the cost of State 2F at 33–36 weeks of gestation. However, these alterations were mainly due to coexisting placental malfunction and poor fetal growth rather than to effects of the drugs per se.

The direct effects of the tocolytics atosiban and nifedipine on fetal functioning were assessed in a randomized study of pregnant women with threatened preterm delivery who also received corticosteroids (de Heus, Mulder, Derks, Kurver, et al., 2008; de Heus, Mulder, Derks, & Visser, 2009). Fetal movements, heart rate (variability), and blood flow parameters measured over a 5-day period did not change during tocolysis in addition to the known effects of betamethasone. Follow-up of children exposed to nifedipine in utero has not demonstrated negative effects on psychosocial and motor functioning at 9–12 years of age (de Heus, Mulder, Derks, & Visser, 2008).

Antidepressants

Selective serotonin-reuptake inhibitors (SSRIs) are commonly utilized to treat maternal anxiety and depression during pregnancy (see Chap. 18 by Hanley, Hookenson, Rurak, & Oberlander). Although their use continues to increase, the clinical efficacy and fetal safety of SSRIs are still a matter of concern (Andrade et al., 2008). SSRIs are stimulatory to the CNS and signals of distress, such as restlessness, jitteriness, and sleep disturbance, are often seen in newborn infants after in utero exposure to SSRIs (Lattimore et al., 2005). These neonatal symptoms may relate to SSRI toxicity or withdrawal at birth, but also to altered CNS development secondary to sustained exposure during fetal life. We recently found evidence of the latter. In an observational ultrasound investigation, fetal behavior was studied at or near 15, 28, and 38 weeks during the pregnancies of women who took SSRIs throughout or who had discontinued medication in early gestation (Mulder et al., 2011). SSRI dosage was categorized as low, standard, or high intake. In fetuses exposed to standard or high SSRI dosages, the %GM at 15 and 28 weeks was higher than in control and low-medicated fetuses. Near term, the prominent finding was disrupted non-REM sleep, caused by continual bodily activity and, thus, poor inhibitory motor control during the quiet state. Figure 5.4 displays the heart rate, GM, and eye movements of a fetus exposed to high levels of maternal SSRI intake. The abnormal continuous GM activity is evident when compared to normal quiet and active periods noted in Fig. 5.3. This unusual and abnormal phenomenon in SSRI-exposed fetuses shows resemblance with a category of adult neurologic disorders called non-REM parasomnias (Chokroverty, 2004). The significance of poor fetal sleep regulation for postnatal neurobehavioral development is cur-

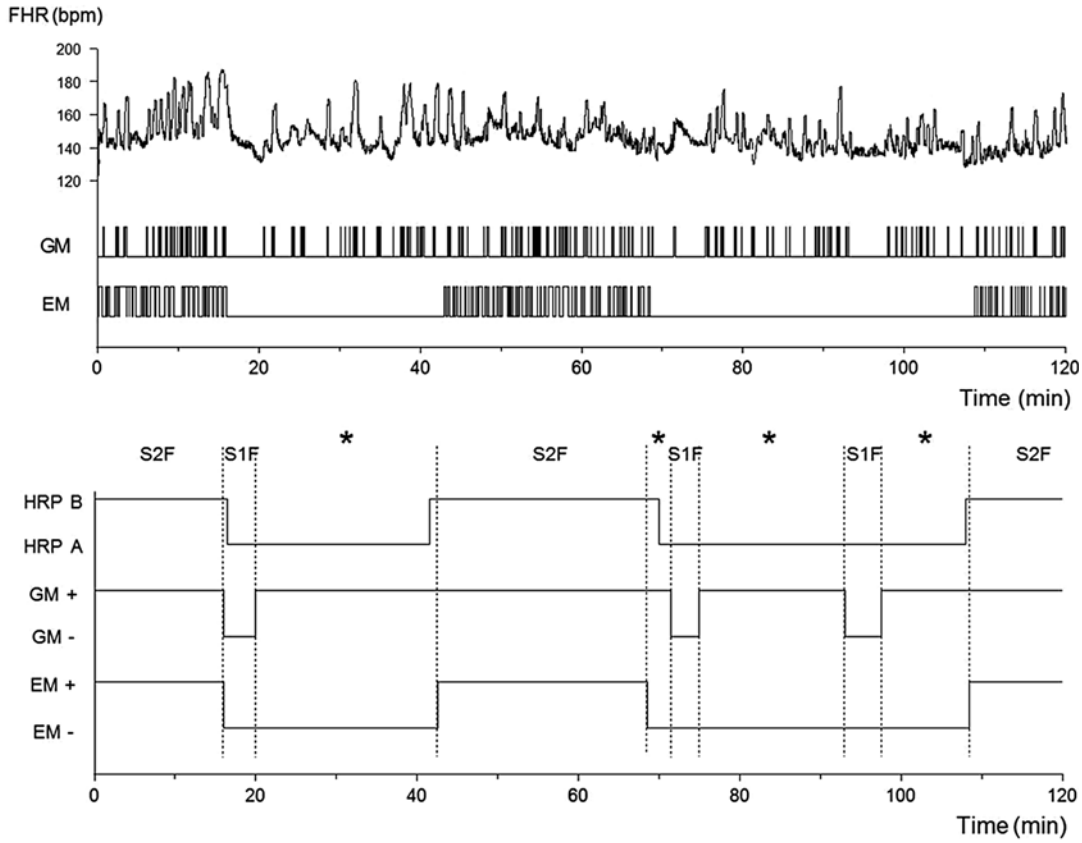

Fig. 5.4 Example of a 2-h fetal behavioral state recording made at 38 weeks of gestation from a pregnant woman with psychiatric problems and long-term use of a high-dose SSRI. Presented are the original data for fetal heart rate (FHR), general movements (GMs), and rapid eye movements (REMs; *upper panel*); and the smoothed state profile indicating episodes of fetal heart rate pattern (HRPs A and B), and the presence (+) and absence (–) of GMs and EMs (*lower panel*). Note disruption of the two 1F combinations (HRPA and absence of EM) through continual presence of GM bursts. S1F: state 1F; S2F: state 2F; *no-state identified. Reproduced from Mulder et al. (2011), with permission

rently unclear and warrants further investigation. Antidepressants are not registered for use during pregnancy which leaves the patient and physician with the question if possible benefits outweigh potential risks. SSRIs may not be prescribed to pregnant women unless in case of severe maternal illness (Visser, Mulder, & Ververs, 2010).

Antiepileptic Drugs

Maternal antiepileptic medication had no demonstrable effect on the quantity or quality of fetal movements nor on fetal state regulation (Kean, Gargari, Suwanrath, Sahota, & James, 2001; Swartjes, van Geijn, Meinardi, van Woerden, & Mantel, 1992). This is in line with postnatal findings. Cognitive function of infants prenatally exposed to commonly used antiepileptic drugs, except valproate, was not impaired at 3 years of age (Meador et al., 2009).

Maternal Stress and Anxiety

Numerous animal studies have demonstrated a plethora of (adverse) effects of prenatal maternal stress/anxiety on physiology, behavior, and cognition in offspring. These studies have considered a multitude of potential underlying mechanisms, including HPA-axis functioning, neurotransmitter systems, and (epi)genetic influences (Huizink,

Mulder, & Buitelaar, 2004). In the human, it is hard to unravel the complex relationship between stress/anxiety during pregnancy and the behavior of an individual before and after birth (Mulder et al., 2002; Van den Bergh, Mulder, Mennes, & Glover, 2005). This is due to a variety of stressor types, measures of stress (physiological vs. psychological), and differences in genetic makeup and coping style on the maternal side, and as to the child, to differences in psychomotor and cognitive test measures at various ages (Buitelaar, Huizink, Mulder, Robles de Medina, & Visser, 2003; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002a). Nevertheless, several authors have found evidence of some association between exposure to prenatal stress and long-term neurodevelopmental sequelae (Gutteling et al., 2005, 2006; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002b, 2003). This paragraph is confined to reporting on two studies performed by us, as the subject is extensively dealt with by others in this book (see Chapters by Glover, Ahmed-de Campos, & Bernardes; and Sandman, Glynn, & Davis). In the first study, maternal emotions were induced by showing a 30-min film of a normal delivery to near-term pregnant women. We found a positive relationship between maternal stress/anxiety levels and the incidence of fetal GM, but no effect on fetal state organization (Van den Bergh et al., 1989). In the second, a longitudinal observational study, fetal behavior was studied at or near 15, 28, and 38 weeks (Robles de Medina, 2004). At these time points, the mothers reported on their level of general stress and pregnancy-specific fears, and salivary cortisol samples were collected from them during the fetal recordings. High levels of general stress were associated with increased fetal GM at all ages, and with less quiet sleep, more GM during REM-sleep, more wakefulness, and an overall better state organization in the fetus near term. However, levels of maternal general stress and cortisol correlated poorly, and the latter was unrelated to the described fetal effects. On the other hand, high pregnancy-specific anxiety and high cortisol levels were found to be associated with reduced fetal motor activity and an increased incidence of quiet sleep. These findings show differential effects on fetal behavior depending on which type of maternal stress/anxiety is being considered (Huizink, Mulder, Robles de Medina, Visser, & Buitelaar, 2004). The data on maternal cortisol nicely fit with those on the effects of administered synthetic corticosteroids (see above) and with those on diurnal variations of FHR variation and fetal body movements. The latter two are highest around midnight and lowest in the early morning (Roberts, Little, Cooper, & Campbell, 1979; Visser, Goodman, Levine, & Dawes, 1982) and are negatively correlated with the maternal cortisol rhythm (Patrick, Challis, Natale, & Richardson, 1979).

Fetal Stimulation and Learning

Fetal Stimulation During Reduced Heart Rate Variability and/or Movements

It is a longstanding belief that evoked fetal responses to external stimulation may differentiate between good and poor condition or may provide insight into fetal brain integrity. Clinically, pregnant women who complain about sensing absent or reduced fetal motility or those whose fetus exhibits prolonged tracings of low heart rate variation during FHR monitoring are abundant. The latter may be indicative of poor fetal oxygenation and/or deterioration. It is conceivable that fetal stimulation has been applied to these cases under the assumption that evoked responses represent CNS reactivity and are a good sign (Tan & Smyth, 2010). However, prolonged episodes of reduced FHR variation are also an important indicator of normal state 1F (quiet) sleep in the healthy third trimester fetus. Several experimental attempts have been made to influence the normal fetus when in quiet sleep. This state remained unaffected by shaking the maternal abdomen (Visser, Zeelenberg, de Vries, & Dawes, 1983), transabdominal sound stimulation (Schmidt, Boos, Gnirs, Auer, & Schulze, 1985), and intense maternal emotions (Van den Bergh et al., 1989). Regularly occurring Braxton Hicks contractions also had no effect and quiet sleep

was even preserved during labor (Griffin, Caron, & van Geijn, 1985; Mulder & Visser, 1987). This indicates that fetal state organization is not easily influenced by external stimuli that may be part of the natural fetal environment and occur in the normal physiological range. Unresponsiveness to common stimulation may be advantageous for the fetus' normal functional development. The findings are in line with those in the newborn that also demonstrate that they are difficult to arouse when in quiet sleep (State 1) (Prechtl, 1974).

Fetal Stimulation to Examine Sensory Sensitivity and Learning

Low-intensity vibro-acoustic stimulation (VAS) devices, such as an electric toothbrush or body massager, can elicit fetal GM and heart rate responses that are immediate and short term (see Chaps. 11 and 8 by Leader and Kisilevsky). However, the use of several different VAS devices has demonstrated that a movement/heart rate response, no response, or a heart rate decrease can be elicited depending on the device employed (Kisilevsky & Muir, 1998). All VAS devices, therefore, are not equal in the response they elicit. One such device that can perturb fetal GM and states is the electronic artificial larynx (Ela), presumably due to its strong and inappropriate stimulus. When we applied an ELa directly to the maternal abdomen, the stimulus induced non-physiological fetal responses: excessive general movements, prolonged tachycardia, and disorganized behavioral states (Visser, Mulder, Wit, Mulder, & Prechtl, 1989). We have advocated not to use this device because it produces intrauterine sound levels beyond 125 dB, and the stimulus may be nociceptive and even harmful to the fetus (Nyman et al., 1991).

Investigators also have studied the effects of repeated fetal stimulation mainly by using an electric toothbrush (see Chaps. 11 and 6 by Leader and Nijhuis). They looked at habituation, defined as the progressive decrease in response when the fetus is stimulated repeatedly. This process is considered a simple form of learning and is supposed to reflect normal brain functioning. However, the importance of habituation in distinguishing between normal and abnormal fetuses is still controversial, mainly because there is no generally accepted standard procedure. Methodological differences among the studies include varying definitions as to habituation, absence of appropriate control episodes during which no stimulation occurred, and neglect of the state dependency of fetal responses to stimulation (Mulder, Robles de Medina, Beekhuijzen, Wijnberger, & Visser, 2001).

Fetal Behavioral States Revisited

Concept and Methodology

In a retrospective view on the emergence of the behavioral state concept in human neonates, Prechtl and O'Brien (1982, p. 54) wrote: "The existence of states is one of those phenomena which, once recognized, becomes so obvious as to make it difficult to understand why it was not appreciated earlier." In the same year, Nijhuis and colleagues described the existence of behavioral states in the near-term fetus (Nijhuis et al., 1982). Subsequently, many investigators, profiting from the year-long experience of others with neonates, took advantage by taking fetal states into account with the interpretation of (clinical) data and the design of research protocols. It is clear by now that the fetal behavioral states (two sleep states and two states of wakefulness) are expressions of discontinuous and distinct modes of neural activity and reactivity; that each state is maintained uninterruptedly over long periods with sharp transitions at the beginning and end, requiring normal brain function; that behavioral (breathing and mouth movements) and physiological variables (micturition, blood flow velocities) show state-dependency; that fetal responsiveness under perturbed conditions also may be state-dependent; that quiet (S1F) and active (S2F) sleep are present for about 30 % and 55 % of the time, respectively, leaving some 15 % for states 3–4F and no-coincidence; that a complete sleep cycle lasts on average for about 90 min with about 30 min and 60 min for S1F and S2F episodes, respectively; and that stud-

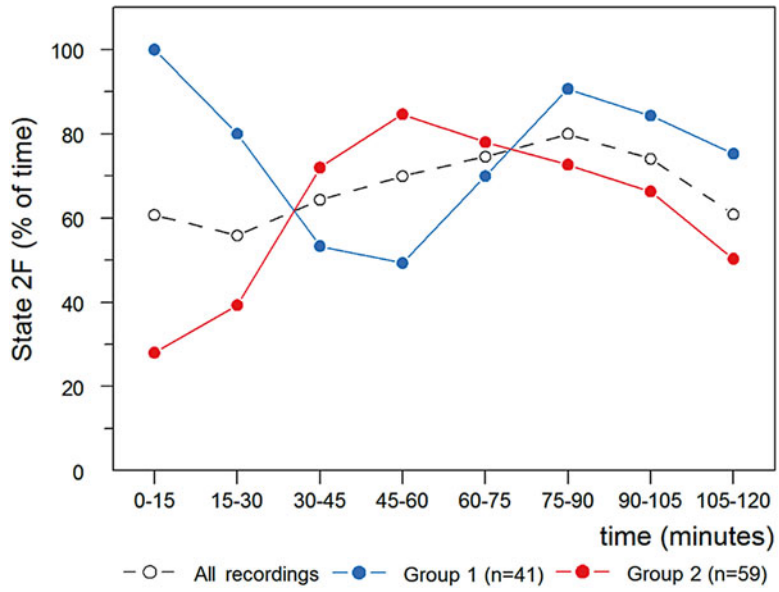

Fig. 5.5 Mean incidence of State 2F (active sleep) in eight 15-min episodes of a 2 h recording for all fetuses ($n = 100$), for fetuses that were in State 2F for the full 15 min of the first episode (Group 1), and for fetuses that were most of the time in State 1F (quiet sleep) during the first episode (Group 2). Mulder, Y. T. B. and Mulder, E. J. H. (unpublished data)

ies of fetal states are time-consuming, especially when waiting for a desired state to occur naturally during which a particular stimulus is to be applied (de Vries & Fong, 2006; Groome et al., 1999; Visser et al., 1992).

All this information has helped to better understand and control inter- and intra-fetal variability in behavioral and physiological measures. However, a large number of fetal studies have appeared over the past 10–15 years that did not consider fetal states properly or even neglected their existence. These studies aimed at soliciting fetal responses brought about by external sound/vibratory stimulation or by acutely elicited maternal stress or emotions during stroop, arithmetic, or temperature challenges. Often, an experiment was composed of a sequence of pretest (control), test, and posttest periods, performed in a relatively short time (<1 h) and with no information about the state the fetus was in during stimulation. Thus, two requirements essential for testing fetal responses experimentally were generally not met: (a) inclusion of a stimulus-free control period of the same duration as the test procedure; (b) guarantee that all fetuses are in the same state when the measurements are made, both when exposed to the stimulation and when unperturbed (control period).

To illustrate how fetal state may influence outcome measures in a stimulation test, we analyzed the distribution of quiet and active states that occurred naturally during the 2-h recordings of 100 healthy unperturbed fetuses at term age (Mulder, Y. T. B. & Mulder, E. J. H., unpublished data). Each recording was divided into eight 15-min episodes. The percentage S2F ranged on average between 60 and 70 % throughout recordings (see Fig. 5.5 for a summary of the %S2F observed over 2 h). Distinction was made between fetuses that presented in active sleep for the full 15 min of the first episode (group 1) versus fetuses that initially spent <15 min in active sleep and were thus in quiet sleep for 1–15 min (group 2). While the %S2F gradually declined over the first hour of recording in group 1, the opposite occurred in the group 2 fetuses, reflecting normal state cycling. Imagine that a 45-min experiment with fetal stimulation at 15–30 min would coincide with the first 45 min in Fig. 5.5 and that group 1 and group 2 fetuses were distributed as shown, erroneous conclusions are self-evident. For group 1, this would include diminished GM and lower FHR variability during and after the fetal challenge, and vice versa for group 2. However, it is easy to see in this example that the results are

due to the condition at the beginning of fetal recording and state cycling, rather than to any effect of fetal perturbation.

Behavioral State Control

Fetal behavioral states can be regarded as precursors of the adult sleep-wake states. Fetal and adult sleep states not only share features of NREM/REM, in terms of stability, rapidity of transitions, and cycle length (80–100 min), but also share the neuronal substrate, neurotransmitters, and receptors that underlie sleep control. Fetal state alternation is likely congruent with the sleep switch (flip-flop) model proposed for adult NREM/REM sleep cycling (Saper, Fuller, Pedersen, Lu, & Scammell, 2010). In this model, either sleep state is controlled by a particular constellation of neurons in pontine, mesencephalic, and hypothalamic centers involving specific neurochemistry. Both state maintenance and transitions result from extensive reciprocal interactions between the two neural constellations. This intricate web of interactions emerges well before birth enabling homeostatic cyclic processes in the immature brain (Scher, 2008). It is therefore conceivable that disturbed fetal state development, for any reason, may have long-lasting effects, including sleep disorders and even mental health problems in later life. However, the study of such long-term sequelae is still in its infancy. There is some evidence that healthy fetuses that had shown synchronized state transitions (<3 min) near term, compared with fetuses that had not, reached a higher level of effortful control at the age of 8 and 15 years (Van den Bergh & Mulder, 2012). This study suggests a link between prenatal regulatory processes and self-regulation in childhood and adolescence.

Concluding Remarks

The scientific interest in the fetus' brain and behavior has a long history (Kisilevsky & Low, 1998). The time that the fetal CNS was thought of as a "bunch of reflexes" is far behind us. Since the advent of ultrasound imaging much has been learnt about spontaneous fetal movement patterns and behavioral states. This knowledge has proven to be useful for understanding physiological phenomena that are of clinical importance for fetal surveillance: FHR patterns and Doppler blood flow velocity profiles. Knowledge of normal fetal behavior also has helped to identify and characterize alterations in behavioral performance and development during pregnancies complicated by disease, drug exposure, or a malformed fetus. Nevertheless, there is no universally accepted procedure to test CNS-functioning in the fetus to date, although there are a number of tests in use (e.g., Biophysical profile score) and being tested (see Chaps. 10 and 11 by Kadic et al. and Leader). In addition, quantitative and qualitative assessment of fetal movements may not be specific enough for identifying the individual fetus with impaired brain functioning, except for isolated cases. Finally, a unifying theory of fetal brain and behavior is lacking.

This is because we are still far from understanding the basic neural mechanisms and functional significance of fetal movements and states. Important questions remain: "Do fetal brain development and behavioral development run parallel during gestation?" and "What exactly does a behavioral abnormality tell us about the fetus' CNS?" We believe a multidisciplinary approach can further our knowledge of prenatal neurology during the most eventful yet the least understood period of our lives. To this end, the recent application of MRI devices for fetal brain mapping is promising. In this way, it is possible to study the structural and functional architecture of neural networks that are involved in fetal movements and sleep organization (see Chaps. 21 and 20 by Schöpf, Langs, & Jakab; and Rousseau, Studholme, Jardri, & Thomason). When combined with the existing knowledge of fetal behavior, fetal MRI will definitely improve insight into CNS structure–function relationships.

With the introduction of new medications, much more attention should be paid to possible effects on fetal behavior and long-term follow-up of infants in utero exposed to these drugs. At present, this is left

to the goodwill of some (poorly financed) research groups. New international guidelines are obligatory and funding of antenatal drug studies needs a much higher priority.

The studies presented in this chapter reflect the scientific journey of two persons who are awed by the mystery of life's beginnings. We have not been on the road to somewhere, we have just been on the road.

We dedicate this chapter with deepest respect to the memory of Prof. Dr. H. F. R. Prechtl (1927–2014) who deceased during the preparation of this review. Heinz was an original thinker and a person of warmth and character. His ideas and work influenced us and many others in profound and positive ways.

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6 Fetal Behavioral and Psychoneurological Development

Jan G. Nijhuis

Abstract

In this chapter, we focus on the assessment of the integrity and activity of the fetal central nervous system (CNS). Therefore it is necessary to understand normal behavior and which variables can be used to assess that (e.g., eye and body movements). Furthermore, one needs to understand the (neuro)developmental pathway during gestation, as a "younger" fetus shows different behavior compared to the "older" fetus. Behavioral states or sleep states are present at the end of gestation, and even wakefulness occurs independent of birth. These states are defined on the basis of three state criteria: cardiotocographic pattern, presence or absence of body, and eye movements.

As in neonatal life, we would like to "test" the CNS, rather than make observations of behavior alone, but we are limited. The intercostal-to-phrenic inhibitory reflex is explained as well as fetal habituation as a measure for fetal memory.

We conclude that it still remains difficult to perform a prenatal neurologic examination. It is quite likely that no single, isolated aspect of behavior alone will evolve to conduct a fetal neurologic investigation, but rather a combination of (behavioral) tests.

Keywords

Fetal behavior • Behavioral states • Cardiotocography (CTG) • Fetal habituation • Intercostal-to-phrenic inhibitory reflex (IPIR) • Fetal neurology • Fetal central nervous system

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Introduction

Over the years, there has been growing interest in the assessment of the integrity and activity of the fetal central nervous system (CNS). Obviously, fetal age is the first and most important issue, as a human fetus shows a clear and fast development of its CNS, not only anatomically, but also if one observes its behavior, a process we call maturation. Second, in neonatal and adult life we can actually "test" the CNS, rather than observe it, but for the fetus this is still very difficult. With the introduction of cardiotocography (CTG) and ultrasonography it is possible to observe and describe normal fetal behavior during gestation. Once normal behavior and its development during gestation have been defined, it also is possible to recognize abnormal behavior or specific changes in fetal behavior. In general, fetal behavior can be studied by investigating and combining fetal heart rate (patterns) and ultrasonic observations of fetal activities such as body movements, eye movements, breathing movements, fetal sucking, and regular mouthing.

For the study of fetal behavior, observations over longer periods of time (e.g., 1–2 h), the use of valid reference ranges appropriate for the gestational age (Nijhuis et al., 1998b; ten Hof et al., 2002) and an objective analysis with strict application of techniques (Nijhuis et al., 1999; ten Hof et al., 1999) are necessary. Without them, comparisons with former or future measurements or between groups of fetuses and studies cannot be made.

In this chapter we will review the most important data on fetal behavioral states and on isolated fetal behavioral variables such as fetal body, eye, breathing, and mouth movements and fetal heart rate (patterns). This will be followed by a description of the first steps in developing tests to assess the fetal CNS, "fetal neurology," and aspects of fetal learning and fetal memory. Whereas I focus on the physiology under normal conditions, Mulder and Visser, in a separate chapter in this book, focus on fetal behavior in complicated pregnancies.

The most important message is that the behavior of a fetus (and thus the fetal condition) can only be properly assessed within the context of gestational age, use of medication by the mother etc. Also, for the neurodevelopment of the fetus, birth by itself is not a substantive issue, as wakefulness and memory are already developed before birth and birth by itself does not lead to a steep rise in developmental pathways.

Fetal Body Movements

Fetal body movements give important information about the condition of the fetus, where, as a rule, the presence of normal movements is associated with a good fetal condition. Ever since the introduction of ultrasound, researchers have focused on both the quantitative and qualitative aspects of fetal movements. More recently, in Norway, it has been shown that giving pregnant women explicit information on fetal movements can reduce stillbirth (Saastad et al., 2010). Most types of movement patterns emerge between 7 and 15 weeks of gestation. From then onwards, 15 distinct patterns can be distinguished (see Table 6.1 for the onset of particular movements). These movements then remain present during the course of pregnancy and their appearance hardly changes (de Vries, Visser, & Prechtl, 1982).

Comparison of quantitative parameters of movements across studies is difficult to achieve. One may count movements, or use a percentage of time the fetus moves, but then a "smoothing technique" is necessary by defining a certain time interval between single movements that constitutes a burst of movement. Early in gestation, this is even more important due to the larger number of movements separated by short intervals, while episodes of fetal quiescence develop gradually, especially after 30 weeks of gestation. Others will use an epoque analysis (e.g., by dividing an observation period of 1 h into 120 half-minutes). These different procedures explain a large part of the discrepancy in results between different studies (ten Hof et al., 1999). The percentage incidence and the num-

Table 6.1 First appearance of several fetal movement patterns in postmenstrual weeks in both singleton and twin gestations (Nijhuis, 2009)

Fetal movement pattern	Singletons	Twins[a]
Fetal heart activity[b]	5.5–6.5	
Just-discernible movement	7.5–8.5	
Startle	8.0–9.5	8.0–10.5
General movement	8.5–9.5	
Hiccup	8.5–10.5	8.0–11.0
Breathing movement	10.5–11.5	9.0–14.5
Hand/face contact	10.0–12.5	8.5–11.0
Jaw opening	10.5–12.5	8.0–12.0
Stretch	10.5–15.5	11.5–15.5
Yawn	11.5–15.5	11.5–15.5
Sucking and swallowing	12.5–14.5	10.0–13.5
Eye movements[c]		
Slow	16.0	
Rapid	23.0	
Movement patterns only in twins[a]		
Touch without reaction		9.5–13.0
First reaction		10.0–13.5
Slow body contact		9.0–16.0
Fast body contact		11.0–15.0
Complex contact: "embrace"		12.0–16.0

Adapted from de Vries et al. (1982), [b]Van Heeswijk et al. (1990), [c]Birnholz (1981), and [a]Arabin et al. (1998)

ber of movements decrease curvilinearly from 24 weeks of gestation. This developmental phenomenon continues with the emergence of fetal rest–activity cycles, which will lead to the recognition of well-developed fetal sleep states or behavioral states near term. Towards term, movements gradually disappear almost completely during these quiet episodes of fetal behavioral state 1F ("non-REM sleep").

Finally, the quality of movements might be a better indicator of the integrity of the fetal nervous system. Normal quality of general movements is defined, for both the prenatal and postnatal periods, as spontaneous, gross movements involving the whole body, lasting from a few seconds to a few minutes, with a variable sequence of arm, trunk, head and leg movements, a waxing and waning in intensity, force and speed, and a gradual onset and end (Prechtl & Einspieler, 1997).

The intrafetal consistency for body movements is low and is probably a feature of the normal development of movements. This high interfetal and intrafetal variation makes the sole measurement of fetal movements, therefore, an inappropriate tool to assess fetal condition. Nevertheless, a percentage incidence of movements below the lower range of normality (2.5–4.0 % after 30 weeks) would warrant further investigations (ten Hof et al., 2002). In daily practice, it is the mother who "knows" how her fetus moves, and concern that the fetus suddenly moves less, is still an alarming signal (Saastad et al., 2010).

Fetal Heart Rate Monitoring and Fetal Heart Rate Patterns

Fetal heart rate (FHR) monitoring is widely used to assess the fetal condition. Normal basal FHR is around 70–80 beats per minute (bpm) at 7–8 weeks of gestation, has a peak of around 180 bpm at 10 weeks of gestation and decreases thereafter (van Heeswijk, Nijhuis, & Hollanders, 1990). In the second and third trimester, the normal basal heart rate is 110–150 bpm (Nijhuis et al., 1998a; Rooth, Huch, & Huch, 1987). In general, good bandwidth or (beat-to-beat) variability and accelerations (in combination with fetal movements) are indicative of a good fetal condition, whereas a persistent silent pattern (small bandwidth, no accelerations) is indicative of fetal distress, certainly in the presence of variable or late decelerations (mostly related to uterine contractions). During gestation, FHR short- and long-term variability and the amount of accelerations increase and, as well, the intensity of the fetal movements increase. There are a number of situations which may induce a silent FHR pattern, see Table 6.2A.

An objective analysis of FHR and FHR short- and long-term variability can be obtained by using a computer (e.g., Sonicaid System, Dawes, Meir, & Mandruzzato, 1994). Although the numerical FHR analysis is preferred over visual analysis which is associated with considerable interobserver and intraobserver variation, in daily practice it is not used in the majority of obstetric units. Nomograms for basal FHR and its long-term (LTV) and

Table 6.2 Differential diagnosis and proposed management in case of a silent fetal heart rate pattern (A) or a sinusoidal fetal heart rate pattern (B)

Differential diagnosis	Management
A. The silent fetal heart rate pattern	
State 1F/FHRP A	Extension of the recording time
Effect of medication	Exclusion of use of medication
Tachycardia	Inspection of the baseline
Anomalies	Ultrasonographic examination
	Behavioral study
Hypoxia	Contraction stress test (CST)
Brain death	Cordocentesis
Very premature fetus	Verification of gestational age
B. The sinusoidal fetal heart rate pattern	
Fetal mouth movements	
Sucking ("major" or "marked")	Behavioral study
Regular mouthing ("minor")	Behavioral study
Effect of medication	Exclusion of use of medication
Congenital anomalies	Ultrasonographic examination
Fetal asphyxia	Biophysical profile testing
Fetal anemia	Cordocentesis

From (Nijhuis, 2009)

short-term (STV) variation show that, with increasing gestational age, basal FHR decreases linearly and FHR-variability increases curvilinearly (Nijhuis et al., 1998a; Ribbert, Fidler, & Visser, 1991). The lower limit (P2.5) of the normal range of FHR-variability increases until 30 weeks of gestation and stabilizes thereafter, despite an overall increase in FHR-variability and a widening of the normal range (Nijhuis et al., 1998b). Furthermore, about 50 % of the differences in FHR-variation can be explained by differences in heart rate (Nijhuis et al., 2000), with FHR and FHR-variation having a negative relationship.

Not only gestation influences the FHR and FHR-variability, but also many other conditions, such as fetal and maternal diseases, medication, fetal diurnal rhythm, cardiac abnormalities, and fetal hypoxia (for an classic overview see Martin, 1978). Furthermore, fetal behavioral states and fetal movements (e.g., regular mouth movements and sucking) can influence FHR and FHR-variation considerably (van Woerden & van Geijn, 1992). Finally, there also is a diurnal rhythm with the highest variability values around midnight, and lowest values in the morning (Visser, Goodman, Levine, & Dawes, 1982).

During pregnancy a progressive patterning of FHR into low and high variation during fetal rest and activity periods, respectively, can be seen. The amount of time spent in low variation gradually increases and, as a consequence, the existence of these rest–activity cycles has to be taken into account when interpreting the FHR tracing (Nijhuis, Martin, & Prechtl, 1984).

Using predefined criteria, FHR patterns (FHRP) can be classified as A, B, C, or D, corresponding to different fetal rest–activity states, the fetal behavioral states 1F to 4F (Fig. 6.1; Nijhuis et al., 1982). Their definitions are as follows: FHRP A is a stable heart rate, with a narrow oscillation bandwidth. Isolated accelerations occur which are strictly related to body movements. FHRP B has a wider oscillation bandwidth with frequent accelerations during movements. FHRP C is stable with a wider oscillation bandwidth than pattern A and there are no accelerations. FHRP D is unstable, with large and long-lasting accelerations, which are frequently fused into a sustained tachycardia: "the jogging fetus." This pattern D might easily be misinterpreted as a "tachycardia with decelerations" if the observer is not alert to the presence of the fetal movements during the recording and its effect on the FHR (Tas & Nijhuis, 1992).

Fetal Breathing Movements and Hiccups

Both fetal breathing movements and fetal hiccups can be observed from 8 to 10 weeks of gestation onwards (de Vries et al., 1982; see Table 6.1). Breathing movements are defined as a paradoxical inward movement of the chest wall with outward movement of the abdominal wall, are considered a normal feature of fetal life and are necessary for the development of the fetal lungs. However, breathing movements are episodic in nature,

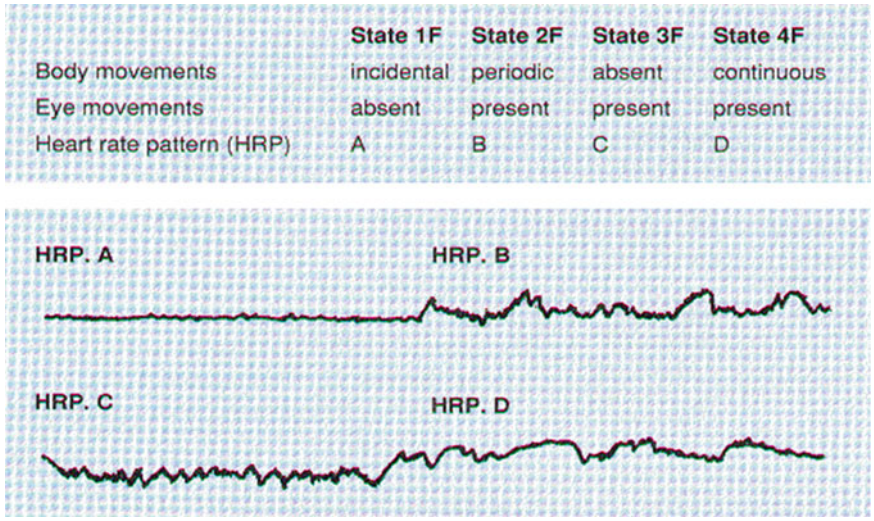

Fig. 6.1 Criteria for fetal behavioral states according to Nijhuis, Prechtl, Martin, and Bots (1982), with examples of the four distinct fetal heart rate patterns (HRP A-D)

subjected to diurnal and ultradian rhythms and influenced by a number of internal and external factors. They are, therefore, highly variable within and between individual fetuses, even under normal physiological conditions (Kisilevsky & Low, 1998). This high interindividual and intraindividual variability makes the use of fetal breathing, as an indicator of fetal health or compromise, of little clinical value, although it is part of the "fetal biophysical profile" (Manning, Platt, & Sipos, 1980). Due to their episodic character, breathing movements cannot be used for the definition of behavioral state, but rather as a state variable.

Fetal hiccups are short and powerful contractions of the diaphragm which can easily be differentiated from breathing movements. In the first trimester, periods with hiccups can be observed very regularly, while in the third trimester only 2–4 episodes with hiccups per 24 h will be noticed.

Fetal Eye Movements

Fetal eye movements (EM) can be observed from 16 to 18 weeks of gestation (Birnholz, 1981; Bots, Nijhuis, Martin, & Prechtl, 1981). Overall frequency of EM increases up to 30–33 weeks, after which the frequency remains constant up to term. From about 30 weeks, consolidation into long-term clusters of EM occurs. Episodes with and without (mainly rapid) EM become closely linked with the other two state variables at about 36 weeks of gestation and then represent behavioral states (Nijhuis et al., 1982).

The frequency of EM is significantly lower in hydrocephalic fetuses than in normal fetuses, while growth-retarded fetuses show less rapid EM (Arduini, Rizzo, Romanini, & Mancuso, 1988). In fetuses with dysmorphic brain structure no EM or EM of a different nature can be found (Birnholz, 1981). Fetuses in breech and cephalic presentations show no difference in the EM incidence, although differences in EM directions are found (Takashima, Koyanagi, Horimoto, Satoh, & Nakano, 1995).

Fetal Mouth Movements

The fetal mouth is easy to visualize using ultrasound; sucking and swallowing can be observed from 12 weeks of gestation onward (see Table 6.1). Recurrent clusters of "regular mouthing movements" can be observed during the quiet state 1F, while during state 3F "sucking movements" can be observed (for an overview, see van Woerden &

van Geijn, 1992). Both regular mouthing and sucking may result in "sinusoidal-like" fetal heart rate patterns which may be confused with underlying pathology like severe fetal anemia (see Table 6.2B; Nijhuis, Staisch, Martin, & Prechtl, 1984b; van Woerden, van Geijn, Caron, van der Valk, & Swartjes, 1988).

Fetal Behavioral States

The combination of the ultrasonographic observation of fetal activity with the simultaneous recording of the FHRP is called the assessment of "fetal behavior." In the first half of pregnancy all movements seem to occur more or less independent of one another and they do not elicit a specific FHRP. In order to recognize "behavioral patterns," linkage of variables (e.g., absence of movements, absence of eye movements and FHRP A) is obligatory (Nijhuis & van de Pas, 1992). This linkage between variables has been described from 25 to 30 weeks (Drogtrop, Ubels, & Nijhuis, 1990), and also at 30–32 weeks (Visser, Poelman-Weesjes, Cohen, & Bekedam, 1987). At about 36 weeks, linkage is such that well developed "fetal behavioral states" can be described: "constellations of physiological and behavioral variables which are stable over time and recur repeatedly, not only in the same infant, but also in similar forms in all infants" (modified from Prechtl, 1969). Three major requirements have to be fulfilled before a behavioral state can be recognized. First of all, a specific combination of certain variables must occur at the same time ("coincidence," "linkage"). Secondly, to be able to recognize this combination, it should be stable over time (by definition at least 3 min). Thirdly, it must be possible to see a clear change from one state into another, a "state transition." By definition, this transition should be completed within 3 min. Based on recordings of fetal behavior with two ultrasound scanners and a simultaneous registration of the FHRP, four behavioral states, 1F through 4F, have been defined (Nijhuis et al., 1982). The suffix "F" for "fetal" was added to indicate the close relationship with the neonatal states defined by Prechtl (1974).

State 1F (similar to state 1 or non-REM-sleep in the neonate): quiescence, which can be regularly interrupted by brief gross body movements, mostly startles. Eye movements are absent. The FHRP "A" is a stable pattern with a small oscillation bandwidth and no accelerations, except in combination with a startle (illustrated in Fig. 6.1).

State 2F (similar to state 2 or REM-sleep in the neonate): frequent and periodic gross body movements—mainly stretches and retroflexions—and movements of the extremities. Eye movements are present. FHRP B has a wider oscillation bandwidth and frequent accelerations during movements (illustrated in Fig. 6.1).

State 3F (similar to state 3 or quiet wakefulness in the neonate): gross body movements absent. Eye movements present. FHRP C is stable but with a wider oscillation bandwidth than FHRP A and no accelerations (illustrated in Fig. 6.1).

State 4F (similar to state 4 or active wakefulness in the neonate): vigorous, continual activity including many trunk rotations. Eye movements are present. FHRP D is unstable, with large and long-lasting accelerations, often fused into a sustained tachycardia (illustrated in Fig. 6.1).

After the introduction of these definitions, many other research groups were able to confirm the same findings (e.g., Arduini et al., 1988; van Vliet, Martin, Nijhuis, & Prechtl, 1985b; van Woerden & van Geijn, 1992). The introduction of the concept of states had a great influence on both animal and human research.

Other behavioral variables appeared to be "state-dependent." As an example, it was shown that breathing movements were largely absent during state 1F (van Vliet, Martin, Nijhuis, & Prechtl, 1985a), but if they were present, they were much more regular (Nijhuis et al., 1983).

In post-term fetuses, Van de Pas, Nijhuis, and Jongsma (1994) showed an increase of the percentage of time spent by the fetus in 3F and 4F, mainly at the expense of state 2F, implying that the fetus was more "awake" in utero.

Furthermore, Doppler measurements in several fetal vessels appeared to be state-related, although in compromised fetuses this state-dependency plays a minor role (van Eyck & Wladimiroff, 1992).

Fetal Wakefulness and Transition to Neonatal Life

In normal post-term fetuses (>41 weeks of gestation), the development of the fetal central nervous system continues, resulting in a significantly increasing percentage of "fetal wakefulness" (i.e., an increasing percentage of states 3F and 4F; van de Pas et al., 1994) at the expense of the percentage of state 2F. The sequence of change of state variables during transitions in these post-term fetuses differs among the various studies. These equivocal results may be due to the generally small numbers of transitions analyzed among the studies and to methodological differences (Nijhuis et al., 1999).

The study by Van de Pas et al. (1994) can be compared with those of Junge (Junge, 1979) who looked at the percentage of wakefulness of newborns, born at 38 and >41 weeks gestational age, respectively, and fetuses of the same age. Fetuses of 38 weeks and babies born at 38 weeks spent the same time in wakefulness, and the same is true for the fetuses of 41–42 weeks and newborns, born earlier but with equal "gestational" ages (see Fig. 6.2, based on data published by Junge in 1979). This proves that wakefulness is not "induced" by being born, but the development of states is a continuum in which birth is an unimportant phenomenon.

As mentioned, fetal behavioral states are comparable with neonatal sleep states and their definitions are similar, although Prechtl, who defined neonatal sleep states 1 through 5, used eyes open or close as a criterion, rather than eye movements, and he also used crying to define state 5 (Prechtl, 1974). Fetal crying, however, has never been shown to be present, although Gingras, Mitchell, and Grattan, in 2005, published an accidental observation of a fetus exhibiting behavior which they explained as "fetal crying." They, therefore, suggest the possibility of a state 5F.

Twins

Multiple pregnancies allow us to analyze specific twin behavior, with passive and active interactions between the two fetuses. When studying twins it must always be noted that the two fetuses are reliably distinguished.

From early gestation onwards, several distinct movement patterns can be demonstrated (see Table 6.1). More complex movements (i.e., combined movements of arms, legs, and body) lasting more than 5 s, emerge somewhat later in pregnancy (Arabin, Mohnhaupt, & Van Eyck, 1998). Investigations into whether these contacts are preferably initiated by one of the multiplets or whether there are differences in the frequency and sensitivity in reaction towards touch, up to now, have not revealed a "dominant" twin (Sherer, Nawrocki, Peco, Metlay, & Woods, 1990). Furthermore, studies could not show differences in the frequency and sensitivity in reaction towards touch between the two twin fetuses and thus no "dominant" position was found. Monochorionic twins are described to have earlier and more numerous contacts, and greater coincidence of behavior. Yet it seems impossible to

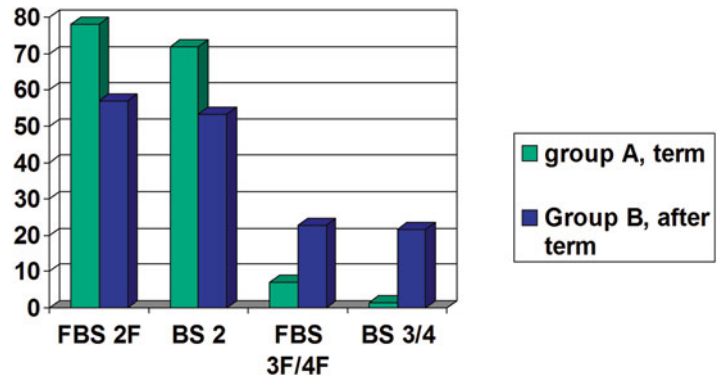

Fig. 6.2 Increasing wakefulness in fetuses (fetal behavioral states, FBS, 3F and 4F) and newborns (behavioral states, BS, 3 and 4) between 38 and 41 weeks at the expense of state 2F in fetuses and state 2 (REM sleep) in newborns (based on data from Junge (1979))

define zygoticity by studying fetal heart rate and fetal behavior in twin pregnancies.

The intertwin contacts have been supposed to cause increased simultaneous activities. So far, conflicting results have been described. In one study, FHR-accelerations were more often associated within the members of a twin pair (57 %) than statistically expected (Sherer et al., 1990), while in another study FHR-accelerations are simultaneous in just 36 %. However, in this last study, synchronous behavioral patterns were exhibited 94.7 % of the time (Gallagher, Costigan, & Johnson, 1992). A third study, assessing fetal movements by ultrasound, demonstrated simultaneous fetal movements only 26 % of the time (Zimmer, Goldstein, & Alglay, 1988).

At advanced gestation, many investigators use the fetal actocardiograph to simultaneously monitor FHR and fetal movements in twins. Without, it is quite difficult to investigate these two parameters simultaneously in two fetuses, needing two separate cardiotocographs and two ultrasound transducers. Yet the use of the actocardiograph might give less reliable results (de Wit & Nijhuis, 2003).

Intertwin differences also are of importance when studying (early) growth differences and twins discordant for fetal anomalies (e.g., Arabin, Bos, Rijlaarsdam, Mohnhaupt, & Van Eyck, 1996; Kurauchi, Ohno, Mizutani, & Tomoda, 1995).

Fetal Neurology

So far, we have focused on fetal behavior, because fetal behavior reflects the activity of the fetal CNS. More direct insight in the fetal CNS and the development of an intrauterine neurological investigation is an important goal in clinical perinatology (see also Nijhuis, 2003).

A global assessment of the fetal condition might be made by using the biophysical profile, where 0 or 2 points are given to each of the five aspects: FHR accelerations, amount of amniotic fluid, fetal body and breathing movements, and fetal tone (Manning et al., 1980). However, the correlation of the biophysical profile with acidemia is much better than with hypoxemia, most likely because the later three aspects only deteriorate late with worsening condition. Furthermore, a state 1F period in a perfectly healthy fetus would get a score of 2 (for normal fluid), because the other criteria would be judged as "abnormal."

So far, direct assessment of the nervous system has been difficult. Fetuses with congenital anomalies may show bizarre behavior or dissociation of heart rate and movements (Tas & Nijhuis, 1992). Other fetuses may show disrupted behavioral states or state transitions, or a delayed development of states (e.g., Mulder, 1993, Nijhuis et al. 1999). Nevertheless, it is still difficult to draw conclusions from a single behavioral recording in a single fetus. In 1993, Tas, Nijhuis, Nelen, and Willems were able to evoke an intercostal-to-phrenic inhibitory reflex (IPIR): compression of the ribcage in a period where fetal breathing is present, results in an apnea. This seemed an interesting approach, but they were not able to find a different result between two groups of normal and growth-retarded fetuses, respectively.

Fetal habituation (i.e., the cessation or decrement of response to a repeated stimulus), is another test. Habituation is a very primitive reflex: it helps to distinguish between "safe" noises and warning signs. For example, if the fetus would not habituate to the environmental sound, it would constantly be stressed by all these "new" sounds, every day. One can compare this with living next to a train station: in the beginning, sleep will be disturbed by the noise of trains, but after weeks or so, one "habituates" and does not hear the trains anymore. For a review into this subject, see Hepper and Leader (1996).

In 2000, Van Heteren, Boekkooi, Jongsma, and Nijhuis (2000a) showed that normal fetuses of 28 weeks and older (and not at 26 weeks) fail to respond after a number of vibro-acoustic stimuli are applied at the level of the fetal leg. And if such a test is repeated within 10 min, this number of stimuli is significantly lower. The fetus therefore seems to recognize the stimulus. This effect also is present if the test is performed after 24 h, indicating that the fetus has a memory and is capable of learning and remembering

(van Heteren, Boekkooi, Jongsma, & Nijhuis, 2000b). This observation appeared to be state-independent (van Heteren, Boekkooi, Jongsma, & Nijhuis, 2001), and in a fetus with an encephalocele, a response could not be evoked (van Heteren, Boekkooi, Jongsma, & Nijhuis, 2000c). Others demonstrated (Hepper & Shahidullah, 1992) that fetuses with Down's syndrome take longer to habituate than normal fetuses.

In another study of normal fetuses from 30 weeks of gestation, Dirix, Nijhuis, Jongsma, and Hornstra (2009) confirmed that, based on habituation to repeated vibroacoustic stimulation, normal fetuses had a short-time memory, because habituation was much quicker when the test was repeated after 10 min. These habituation tests were then repeated at 38 weeks and compared with fetuses of 38 weeks who had not been exposed to the vibroacoustic stimulus before. Fetuses who were already tested at 34 and 36 weeks respectively, showed a significantly faster habituation rate at 38 weeks than the unexposed fetuses, indicating that these fetuses already had a 4-week's memory (Dirix, Nijhuis, et al., 2009). The same group also wondered if there was a relation between habituation rate and the early essential polyunsaturated fatty acid status (ePUFA) of the mother, which is considered of great importance for brain development and function (Innis, 2007). They did find a weak but not significant difference. They concluded that the availability of these fatty acids probably do not determine the differences in these primitive brain functions during the third trimester of fetal development (Dirix, Hornstra, & Nijhuis, 2009).

Hepper used an acoustic stimulus which elicits a "fetal startle response," as a possible means to test fetuses neurologically. This startle response appears to be present from 26 to 27 weeks of gestation. They also argued that, for this test, fetal hearing must be present (Hepper & Shahidullah, 1994). In a recent study they looked at this response in fetuses of mothers who used alcohol during pregnancy. They showed that alcohol delays the emergence of the fetal elicited startle response, but only transiently: the delay was present at 29 weeks of gestation, but had appeared in fetuses at 32 weeks of gestation. (Hepper, Dornan, Lynch, & Maquire, 2013).

In a recent review in 2013, Hepper discussed the developmental origin of laterality (fetal handedness), which is also a fundamental feature of intrauterine neurologic development. This is another example of central aspects of human fetal behavior. It emphasizes again that the neurodevelopmental pathway of the CNS really starts prenatally and continues into neonatal life after birth.

Conclusion

It must be clear that insight into fetal behavior is crucial for the understanding of normal fetal well-being and in the evaluation of the possibly compromised fetus. It has introduced a completely different way of looking at the developing human being. However, it is very time-consuming to study fetal behavior as a routine screening method. Furthermore, although the patterns of development of fetal behavior exist for most healthy fetuses, there is a wide normal range which makes the identification of the compromised fetus very complex (Nijhuis et al., 1999). Therefore, it is difficult to differentiate between normal and impaired neurological maturation. These large normal ranges are mainly caused by considerable interindividual differences, but the influence of factor(s) complicating pregnancy (maternal and fetal) and drug-induced effects also play a role in this. Much more investigation is therefore needed to unravel the (possible) effects of specific drugs on human fetal behavior.

We also conclude that it still remains difficult to perform a prenatal neurologic examination. It is quite likely that no single, isolated aspect of behavior alone well evolve to conduct a fetal neurologic investigation, but rather a combination of (behavioral) tests.

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Linear and Nonlinear Analysis of Fetal Heart Rate Variability

7

Hernâni Gonçalves, Diogo Ayres-de-Campos, and João Bernardes

Abstract

Cardiotocography (CTG) is widely used in pregnant women for fetal monitoring and has provided extensive knowledge on the pathophysiology of fetal hypoxia. Computer analyses of CTG tracings were developed to reduce intraobserver and interobserver disagreement inherent to visual analyses, but commonly rely on the use of simple linear time-domain indices, providing a limited understanding of fetal heart rate (FHR) variability. Additional information can be obtained from linear analysis of FHR in the frequency-domain and from nonlinear methods such as measures of entropy. Such indices are able to characterize fetal behavioral states, different gestational ages, and to identify situations of fetal acidemia. However, the technical specifications of fetal monitoring equipment and fetal presentation should be considered in order to ensure an adequate application of these indices. In addition to these, the inclusion of fetal gender may improve the identification of fetal acidemia and intrauterine growth-restriction. In conclusion, linear and nonlinear analysis of FHR variability is likely to improve the performance of existing computer systems for CTG analysis, justifying more extensive clinical trials with these methods.

Keywords

Cardiotocography • Fetal monitoring • Computerized analysis • Fetal heart rate • Signal processing • Variability • Spectral analysis • Entropy • Fetal behavioral states • Acidemia

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Introduction

Cardiotocography (CTG), a medical technology which consists of the continuous recording of fetal heart rate (FHR) and uterine contraction signals, was introduced into clinical practice about 40 years ago, and is now the most widely used fetal monitoring technique in industrialized countries. It can be used both in the antepartum and intrapartum periods, and has provided extensive knowledge on the pathophysiology of fetal hypoxia. The most prominent scientific associations recommend the surveillance of selected pregnancies from 24 to 26 weeks of gestation to term, and fetal monitors are widespread in modern obstetric units (ACOG, 2005; FIGO, 1995; RCOG, 2001; Rooth, Huch, & Huch, 1987).

Visual analysis of CTG tracings is associated with a high intra- and inter-operator disagreement (Ayres-de-Campos, Bernardes, & Costa-Pereira, 1999), and this has led to the development of systems for computer analysis of CTG tracings that are able to provide real-time alerts for healthcare professionals when changes associated with fetal hypoxia are detected (Nunes, Ayres-de-Campos, Figueiredo, & Bernardes, 2013). One of such systems is the Omniview-SisPorto® (Ayres-de-Campos, Sousa, Costa, & Bernardes, 2008), from which a screenshot is shown in Fig. 7.1. These systems commonly rely on the use of simple linear time-domain indices, providing only a limited understanding of all the information contained in the FHR signal and thus reducing its contribution to the improvement of perinatal indicators (Nunes et al., 2013).

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Additional information can be obtained through the use of indices derived from linear analysis in the frequency-domain (spectral analysis) (Oppenheimer & Lewinsky, 1994), and nonlinear methods such as entropy measures (Pincus & Viscarello, 1992). Substantial research has been published on the application of these alternative linear and nonlinear FHR indices and they have proven to be useful in the detection of low umbilical artery blood (UAB) pH (Gonçalves, Rocha, Ayres-de-Campos, & Bernardes, 2006b), particularly in intrauterine growth restricted fetuses when additional information such as the gender of the fetus is considered (Gonçalves, Bernardes, & Ayres-de-Campos, 2013).

In this chapter linear (time- and frequency-domain) and nonlinear indices (entropy and compression) not commonly considered in computer systems for analysis of FHR variability are briefly described as well as the influence that technical specifications have on the computation of these indices. This is followed by an explanation of how these indices vary with respect to fetal behavioral patterns, gestational age, fetal presentation, and acidemia, with special reference to the importance of considering fetal gender in several of these situations. Current developments on the analysis of the fetal electrocardiogram (FECG) will be briefly addressed.

Methods of Fetal Heart Rate Variability Analysis

Current research on the analysis of FHR variability comprises the computation of linear and nonlinear indices, on a given FHR signal $x(i)$ with N signal points ($i=1,\ldots,N$). Linear time-domain indices usually considered include mean FHR (mFHR), standard deviation of FHR (sdFHR), long-term irregularity index (LTI), Delta FHR (Δ), short-term variation (STV) and interval index (II), which are defined in the following equations:

$$\mathrm{mFHR} = \overline{x} = \frac{1}{N}\sum_{i=1}^{N} x(i)$$

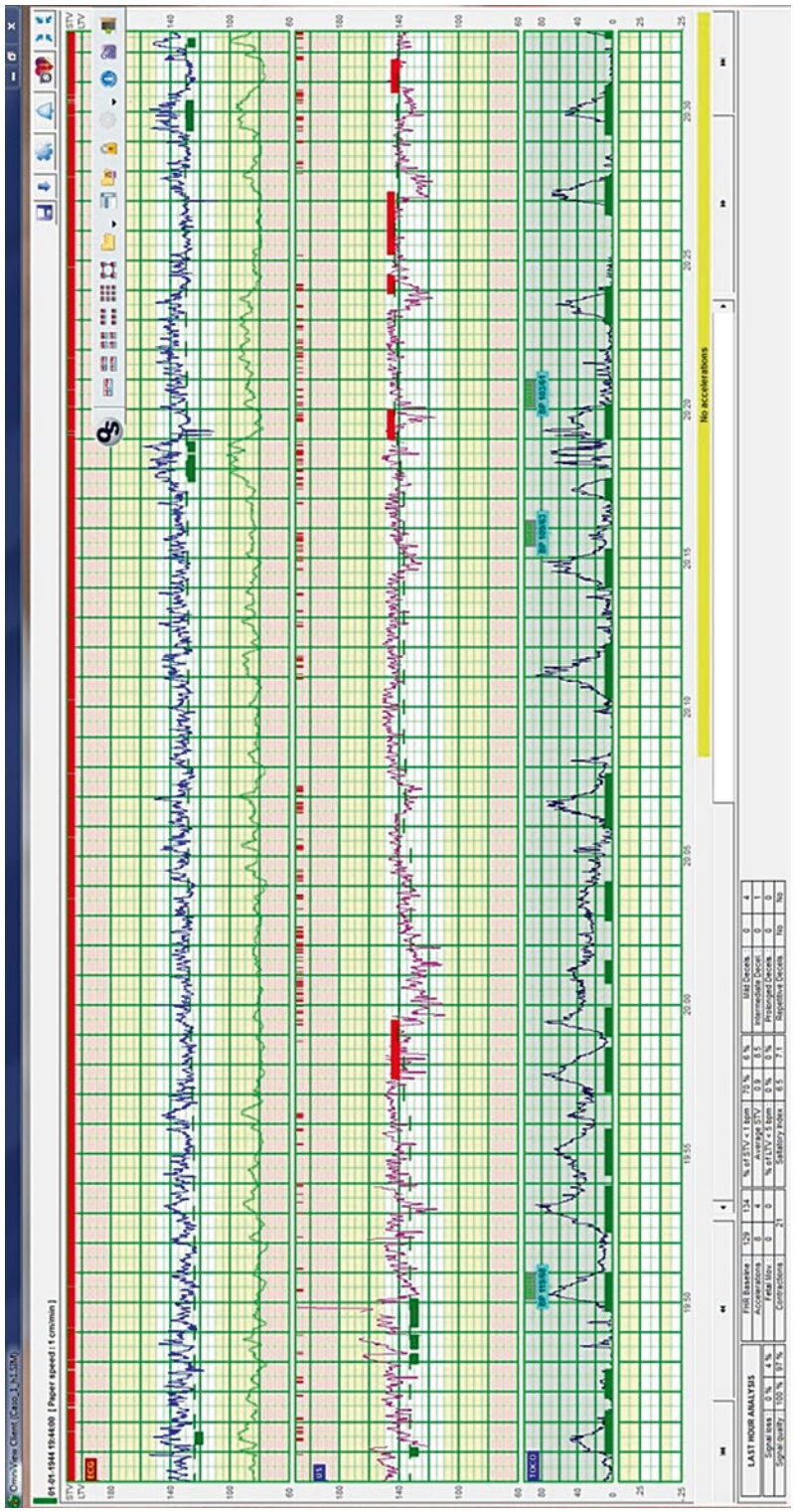

Fig. 7.1 Example of the Omniview-SisPorto® system for computer analysis of CTG tracings. The two *upper* tracings correspond to the maternal heart rate (obtained from ECG and pulse oximetry), the *middle* tracing corresponds to the FHR and the *lower* tracing to the uterine activity. A table with several features computed from the last hour of analysis is given at the *bottom*. Further details can be found in Ayres-de-Campos et al. (2008), Pinto et al. (2014)

$$\text{sdFHR} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(x(i)-\bar{x}\right)^2}$$

$$\text{LTI} = \text{IQR}\left(\sqrt{x^2(i)+x^2(i+1)}\right)$$

$$\Delta = \frac{1}{M}\sum_{i=1}^{M}\left[\max_{i\in M}\left(x(i)\right)-\min_{i\in M}\left(x(i)\right)\right]$$

$$\text{STV} = \frac{1}{24M}\sum_{i=1}^{24M}\left|\text{sm}(i+1)-\text{sm}(i)\right|$$

$$\text{II} = \frac{\text{STV}}{\text{STD}\left[\text{sm}(i)\right]}$$

where IQR denotes the interquartile range (with $i = 1, \ldots, N-1$), M is the number of minutes of the signal, sm(i) are the values of $x(i)$ on each period of 2.5 s, and STD represents the standard-deviation. All these indices but II reflect gross changes in FHR average and variability, whereas II assesses short-term FHR variability taking into account long-term variability (Gonçalves, Rocha, Ayres-de-Campos, & Bernardes, 2006a).

For frequency domain linear analysis, the following frequency bands are commonly considered (Signorini, Magenes, Cerrutti, & Arduini, 2003): very low frequency (VLF) at 0–0.03 Hz, low frequency (LF) at 0.03–0.15 Hz, movement frequency (MF) at 0.15–0.50 Hz, and high frequency (HF) at 0.50–1.00 Hz. LF and HF are mainly associated with activity of the sympathetic and parasympathetic systems, respectively, whereas the MF band pertains to fetal movements and maternal breathing. LF/HF and LF/(MF + HF) reflect the balance between the sympathetic and parasympathetic branches of the autonomic nervous system. An example of a FHR segment and its spectrum is given in Fig. 7.2.

A traditional nonlinear approach that is widely known is the Poincaré plot, which consists of the representation of time series points versus its preceding points (also called a recurrence map). The short- and long-term variability indices are computed from the Poincaré plot, respectively SD_1 and SD_2, as well as the ratio SD_1/SD_2 (Task Force, 1996).

Other commonly used nonlinear indices are approximate entropy, ApEn(m,r) (Pincus & Viscarello, 1992), and sample entropy, SampEn(m,r) (Richman & Moorman, 2000), which measure the amount of irregularity/complexity present in a signal. Usually, the values 0.1 STD, 0.15 STD, and 0.2 STD are adopted for the threshold r, whereas the embedding dimension m is usually considered as 2 (Pincus & Viscarello, 1992). An example comprising a periodic and a random sequence is shown in Fig. 7.3, where more complexity leads to higher entropy values. Multiscale entropy (MSE) analysis is an approach which incorporates the concepts of entropy and scale (Costa, Goldberger, & Peng, 2002; Costa, Goldberger, & Peng, 2005), and consists of dividing the original time series into nonoverlapping windows of length τ, followed by averaging the data points inside each window. An entropy measure (usually SampEn) is calculated for each coarse-grained time series plotted as a function of the scale factor τ.

Recently, it also was shown that compression, another nonlinear measure rarely used in FHR,

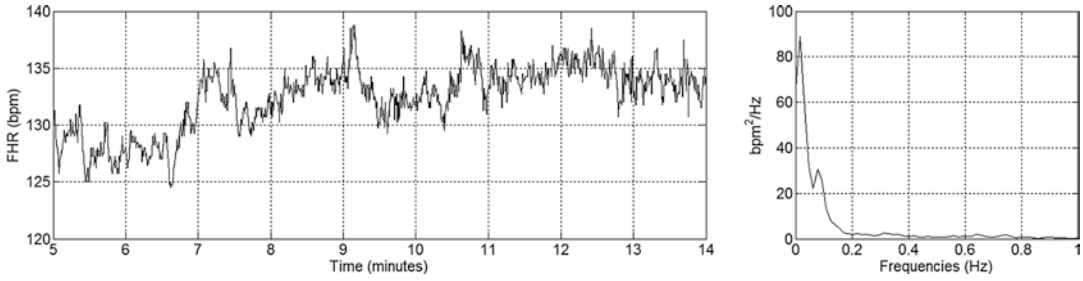

Fig. 7.2 An example of a FHR segment (*left plot*) and its corresponding spectrum (*right plot*)

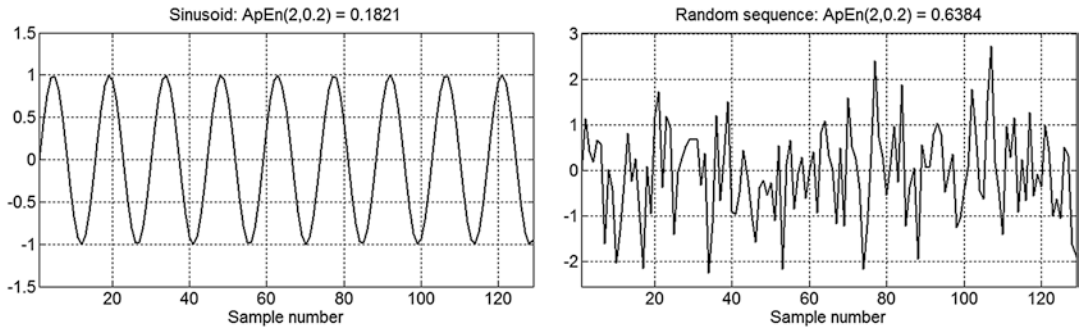

Fig. 7.3 Examples of a periodic (*left plot*) and a random sequence (*right plot*) signal. The periodic sequence displays much less irregularity than the random one. Their corresponding ApEn(2,0.2) values are 0.1821 and 0.6384, respectively

can be used as an alternative to entropy in the quantification of complexity in biological signals (Henriques et al., 2013). By using entropy and compression approaches, one can quantify different features of system complexity, as shown by the low/moderate correlations between entropy and compression measures (Henriques et al., 2013). Entropy and compression indices also have the advantage of requiring short computational times, thus allowing them to be included in systems for computer analysis of the FHR.

The mentioned linear and nonlinear indices provide complementary information on the characterization of FHR variability and thus different features should be considered to obtain a proper evaluation of the fetal state (Gonçalves, Bernardes, et al., 2013, Gonçalves et al., 2006b).

Influence of Technical Specifications of the Monitoring Equipment

Technical specifications of the monitoring equipment, namely the mode of acquisition (internal or external) (Gonçalves et al., 2006a) and sampling/quantization parameters (Gonçalves, Bernardes, et al., 2013), may significantly influence the computation of FHR variability indices and should thus be taken into account, particularly in the definition of reference intervals.

FHR signals can be acquired externally and noninvasively using an ultrasound sensor placed on the mother's abdomen, or alternatively they can be acquired internally using a scalp electrode introduced through the vagina and attached to the fetal scalp (typical FHR tracings are presented in Fig. 7.4). The latter can only be used during labor, after the beginning of cervical dilatation and rupture of the fetal membranes.

In a study based on a sample of 33 normal singleton term pregnancies monitored in the last 40–60 min before vaginal delivery, FHR signals were simultaneously acquired with external and internal modes, using the two FHR channels of a Hewlett-Packard M1350A or M1351 monitor, at a sampling frequency of 4-Hz (Gonçalves et al., 2006a). Most time domain linear indices were similar with external and internal monitoring in the initial and final segments of the tracings. However, linear frequency domain indices were poorly correlated in the final segments and had significantly different mean values in the initial segments. Nonlinear indices were significantly different in both initial and final segments (Gonçalves et al., 2006a). Therefore, the mode used to acquire FHR signals can significantly affect the ability to assess FHR tracings in the final minutes of labor, particularly with linear frequency domain and nonlinear indices.

In another study comprising 27 intrapartum FHR tracings obtained in singleton term fetuses in the last hour of labor using a STAN®21 monitor (Neoventa®, Gothenburg, Sweden) and a scalp electrode, the dataset was divided into two groups according to the UAB pH. Twenty-one cases had an UAB pH $\geq$ 7.20 and six had an UAB pH $<$ 7.20

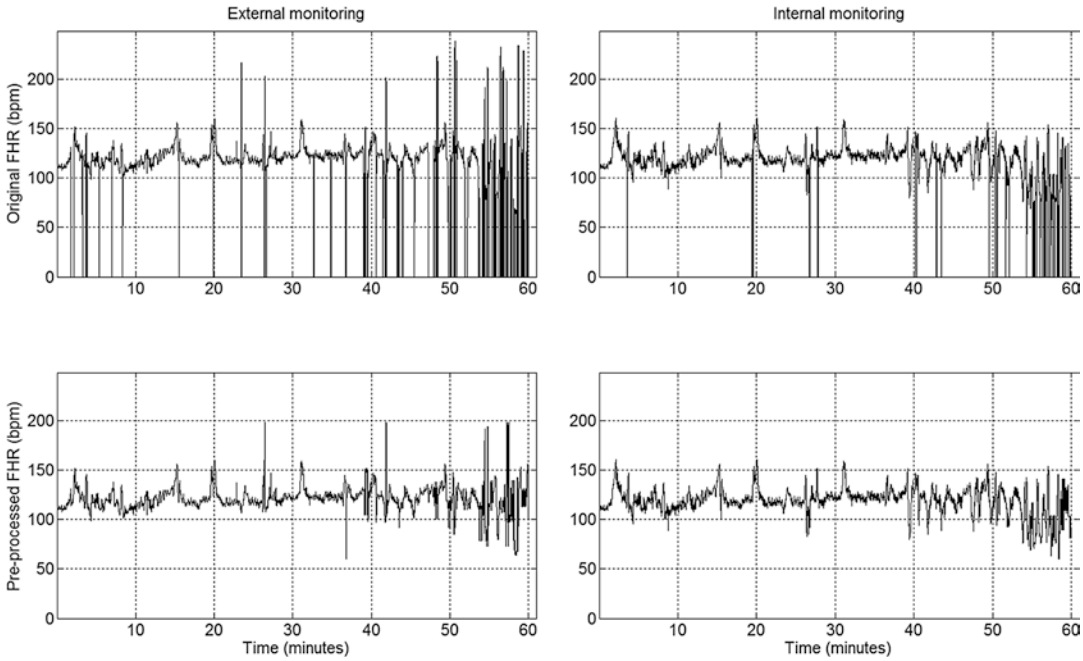

Fig. 7.4 Simultaneous external (*upper left*) and internal (*upper right*) recordings of FHR signals from the same fetus in the 60 min preceding delivery, and corresponding tracings (*lower plots*) after the application of the pre-processing algorithm described in Gonçalves et al. (2006a)

(Gonçalves, Costa et al., 2013). Four different combinations of sampling/quantization specifications were compared: original beat-to-beat signals in milliseconds converted to bpm (rr), beat-to-beat integer values in bpm (hr), after 4-Hz sampling and after 2-Hz sampling. Significant differences in variability indices were found between beat-to-beat and 4-Hz sampled signals, with a lesser effect seen with 2-Hz sampling. These differences did not affect the physiological changes observed during labor progression, such as decreased entropy and linear time domain indices, and increased frequency domain indices. However, significant differences were found in the discrimination between fetuses born with different UAB pHs, with beat-to-beat sampling providing better results in linear indices and 4-Hz sampling better results in entropy indices (Gonçalves, Costa et al., 2013). In conclusion, sampling/quantization specifications, in addition to the acquisition mode, must be taken into account when interpreting, defining, and comparing values for FHR indices.

Fetal Behavioral States

The occurrence of different behavioral states is a hallmark of fetal neurological responsiveness and absence of hypoxia/acidosis. These states correspond to calm or no-eye movement (NEM) sleep (state 1F), active or rapid eye movement (REM) sleep (state 2F), calm wakefulness (state 3F) and active wakefulness (state 4F). They are important for interpreting FHR monitoring, as well as for diagnosing pathological fetal conditions (Nijhuis & ten Hof, 1999). Fetal behavioral states emerge and may be recognizable as early as 30–32 weeks gestational age and are usually well organized and display clear state transitions by 34–36 weeks, but this does not generally occur in fetuses with severe hypoxia and/or growth restriction, central nervous system malformations and brain death (Vindla & James, 1995). FHR pattern A is associated with fetal state 1F and has a stable baseline with absent or only sporadic and short-lasting accelerations. Pattern B is associated with fetal state 2F and is the

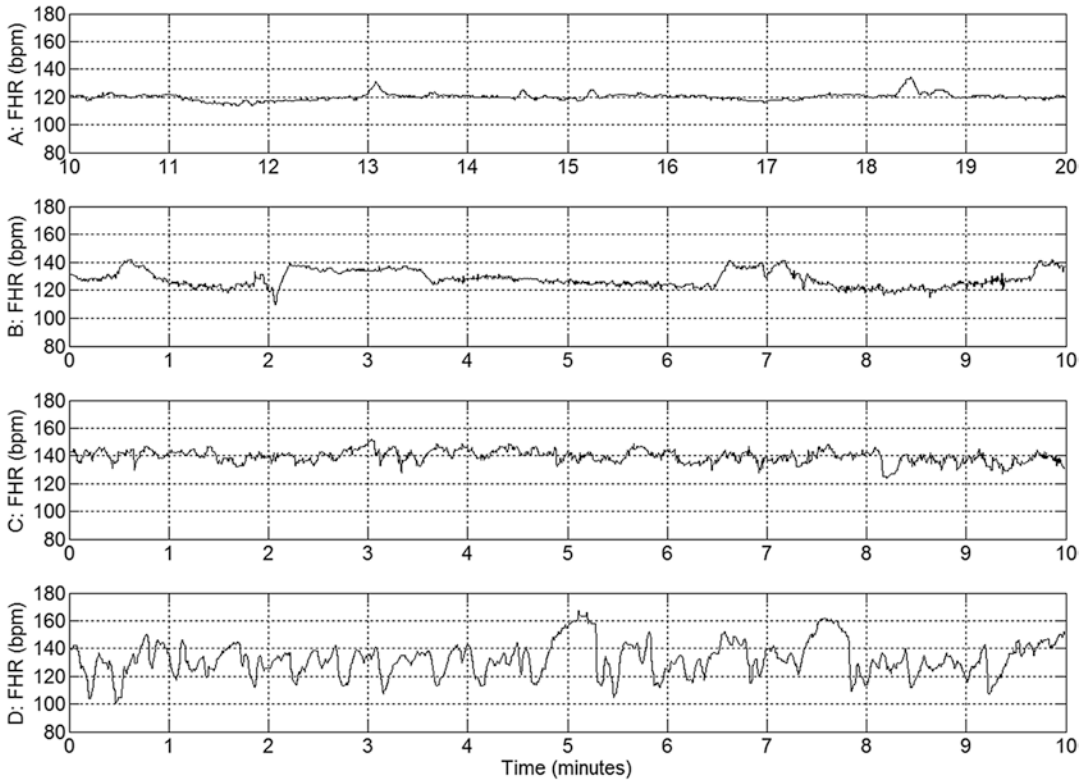

Fig. 7.5 Typical examples of 10-min FHR segments classified as patterns A, B, C and D

most frequently encountered. It displays a stable baseline and frequent accelerations. Pattern C, associated with fetal state 3F, is the rarest and has the shortest duration. It displays a stable baseline like pattern A, but a wider variability and no accelerations. Finally, pattern D is associated with fetal state 4F, showing repetitive and frequently long-lasting accelerations with brief returns to the baseline (Swartjes, van Geijn, Mantel, van Woerden, & Schoemaker, 1990). Recently, a fetal autonomic brain age score based on these heart rate patterns was proposed, composed by time- and frequency-domain indices computed from fetal diagrams (Hoyer et al., 2013). Typical examples of each pattern are presented in Fig. 7.5.

Fifty FHR tracings from normal term pregnancies were acquired in the antepartum period, using external monitoring at a sampling rate of 4-Hz followed by 2-Hz resampling (Gonçalves, Bernardes, Rocha, & Ayres-de-Campos, 2007). Most linear domain indices increased significantly with rising fetal activity whereas the opposite occurred with nonlinear indices, except for SampEn(2,0.1). LF/(MF+HF) ratio also increased significantly with fetal activity, denoting a higher sympatho-vagal balance. FHR patterns associated with active sleep (B) and active wakefulness (D) evidenced more signs of autonomic nervous system activity, with sympatho-vagal imbalance, and less signs related to complexity or irregularity control systems than patterns associated with calm sleep (A) and calm wakefulness (C) (Gonçalves et al., 2007). Additionally, sleep patterns (A and B) presented a higher irregularity than the active patterns (C and D).

Gestational Age

Gestational age is another factor to be considered in the analysis of FHR variability, due to the different developmental stages of the fetal autonomic and central nervous systems. In studies based on fetal magnetocardiography, which is not used in clinical practice but provides a more accurate eval-

uation of FHR variability, a significant influence of gestational age on linear and nonlinear indices was found (Lange, Van Leeuwen, Geue, Hatzmann, & Grönemeyer, 2005; Van Leeuwen, Lange, Betterman, Grönemeyer, & Hatzmann, 1999).

A prospective cohort study was conducted in 50 singleton pregnancies with FHR recorded from the 24th week of gestation onwards, using external monitoring at a sampling rate of 4-Hz (Amorim-Costa, Gonçalves, Bernardes, & Ayres-de-Campos, 2012). An average of 5.4 recordings were performed in each fetus. Significant differences were found throughout pregnancy for all entropy indices, associated with a progressive increase from the 24th week to the 38th week, and a decrease thereafter. A rise in sympatho-vagal balance (LF/(MF + HF)) also was verified after 26 weeks, followed by a slight decrease after the 35th week (Amorim-Costa et al., 2012). Figure 7.6 illustrates the evolution of mFHR, LF/(MF + HF) and SampEn(2,0.1) throughout pregnancy.

Spectral and entropy analysis of FHR signals were thus able to identify an increase in sympatho-vagal balance and in entropy indices between 24 and 38 weeks followed by a decrease from then onwards. This reinforces the need to consider gestational age on FHR analysis in order to evaluate the fetal condition with greater accuracy.

Fetal Presentation

The type of fetal presentation is associated with differences in intrauterine neurodevelopment and consequently in post-neonatal perceptual and learning capabilities (Sørensen et al., 1999; Van der Meulen, Davies, & Kisilevsky, 2008). Additionally, it also can influence the criteria used to define normality in fetal behavior and related variables, such as the FHR (Kean, Suwanrath, Gargari, Sahota, & James, 1999; Park, Ryu, Shim, Hoh, & Park, 2012). Studies of fetal behavior involving ultrasonographic monitoring of different movements are complex, time-consuming, and technically difficult to conduct. Linear and entropy analysis of FHR recordings may provide a simpler alternative, allowing an evaluation of the autonomic nervous system, and that of the more complex physiological control systems (Gonçalves et al., 2007).

A recent study evaluated 11 breech presentations matched for gestational age, weight and gender with 16 cephalic controls (Gonçalves, Ayres-de-Campos, & Bernardes, 2014). All cases had uneventful singleton pregnancies, were scheduled for an elective Cesarean section, had normal fetal outcomes, and FHR tracings were acquired with external monitoring at a beat-to-beat sampling rate. Breech presentations exhibited significantly higher mFHR, sdFHR, LTI, and LF/(MF + HF), but lower II, ApEn, SampEn, and r_{Lu}, than their cephalic counterparts. Differences were more obvious among males (mFHR, sdFHR, LTI, II, ApEn, and SampEn) than among females (II and r_{Lu}), matched for gestational age, weight and Apgar scores (Gonçalves et al., 2014). Breeches denoted increased sympatho-vagal balance and decreased complexity, consistent with the occurrence of more active fetal behavioral states.

These findings suggest that there are neurodevelopment differences between fetuses in cephalic and breech presentation, and further research is needed to confirm if these have important implications in the development of postnatal perceptual and learning capabilities, as well as in the evolution of other breech associated behaviors (Fong, Savelsbergh, Leijsen, & de Vries, 2009). Additionally, systems for computer analysis of the FHR should consider the possibility of a special configuration for the evaluation of breech presentations.

Acidemia

FHR monitoring is frequently used during labor and it can result in immediate obstetric intervention because of life-threatening situations related to fetal acidemia (ACOG, 2005; RCOG, 2001; Rooth et al., 1987). Despite technical difficulties such as high signal loss and noise-to-signal ratio during the final minutes of labor, making tracing interpretation a complex task, some attempts at computerized analysis of the FHR have been performed, with promising albeit preliminary results (Chung et al., 2001; Gonçalves et al., 2006b; Salamalekis et al., 2002).

In a study evaluating 68 fetuses, of which 48 had normal UAB pH ($pH \geq 7.20$), 10 were mildly

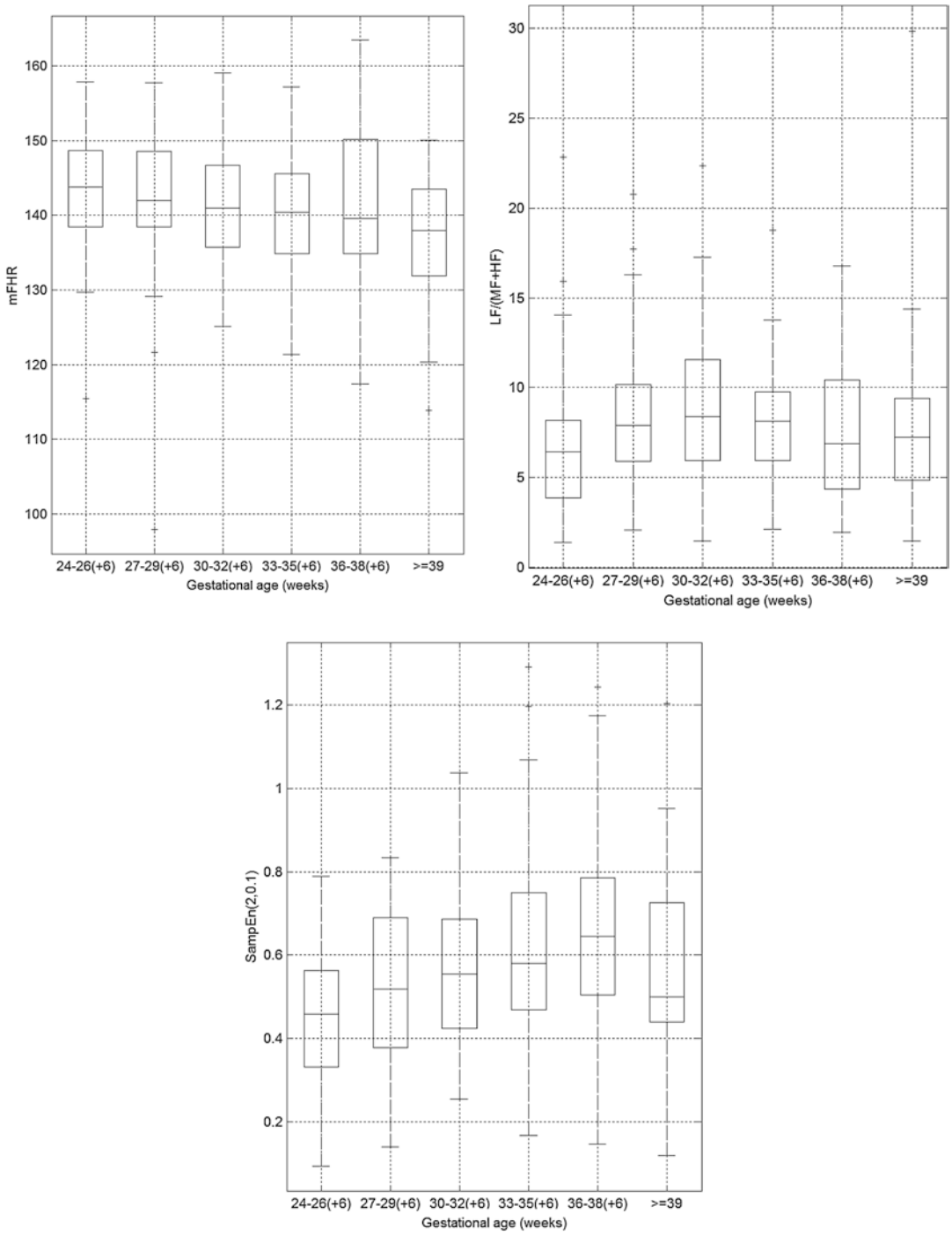

Fig. 7.6 Evolution of mFHR, LF/(MF + HF) and SampEn(2,0.1) throughout gestation

acidemic (7.10<UAB pH<7.20) and the remaining 10 were moderate-to-severely acidemic (UAB pH≤7.10), FHR tracings were acquired with internal monitoring in the last hour before delivery, at a sampling rate of 4-Hz followed by 2-Hz resampling (Gonçalves et al., 2006b). Progression of labor was associated with a significant increase in linear frequency domain indices whereas nonlinear indices were significantly decreased. Moderate-to-severe acidemia was associated with a significant decrease in nonlinear indices. The best discrimination between moderate-to-severe acidemic fetuses and the remaining cases was obtained combining II and ApEn(2,0.15), showing a specificity of 71 % and a sensitivity of 80 % (Gonçalves et al., 2006b). The distribution of indices II and ApEn(2,0.15) for the three groups in the initial and final segments of the tracing is shown in Fig. 7.7.

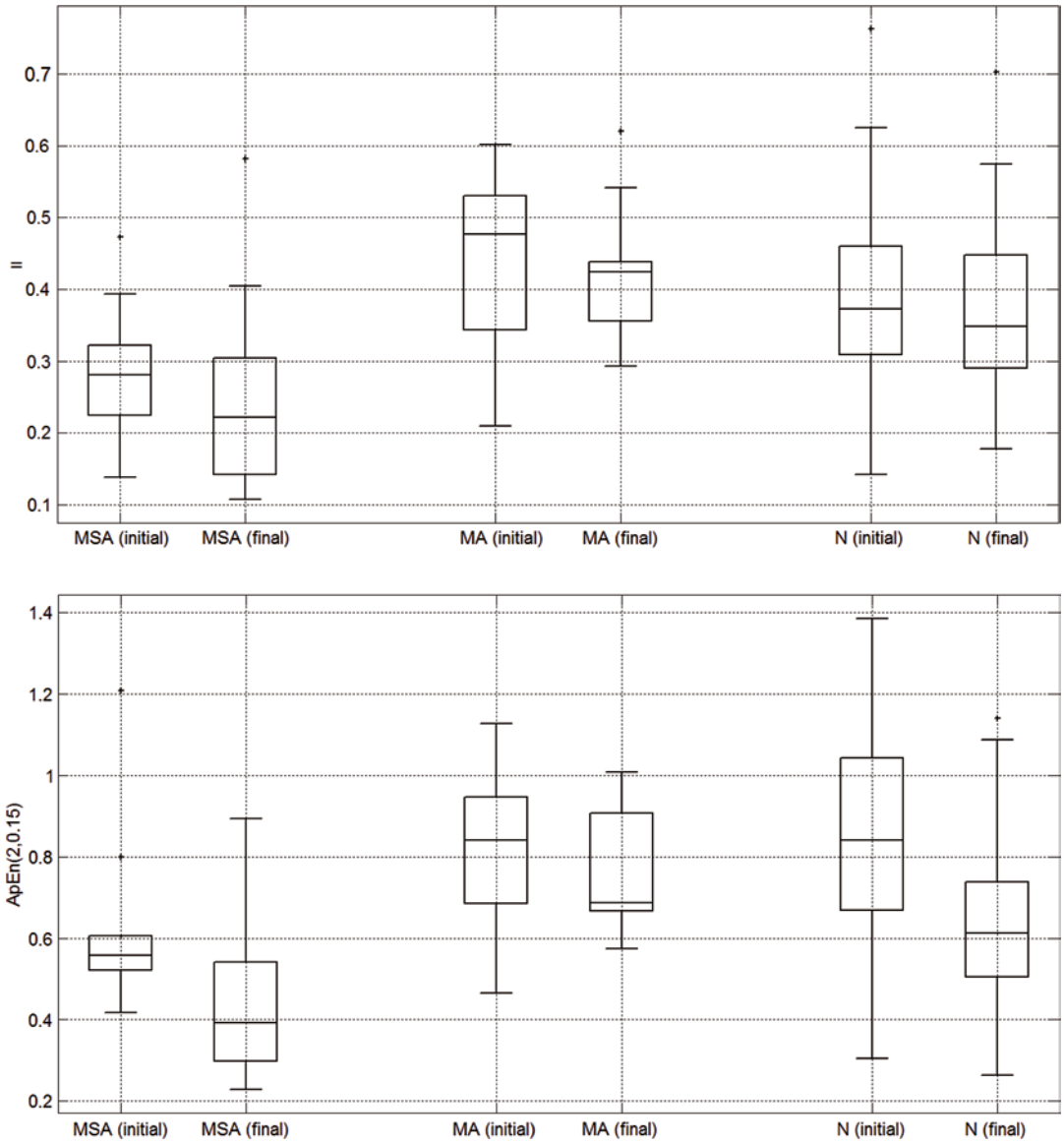

Fig. 7.7 FHR indices II and ApEn(2,0.15) for the moderate-to-severe acidemic (MSA), mildly acidemic (MA) and normal uterine artery blood pH (N) groups, in the initial and final segments of the minutes preceding delivery. Further details can be found in Gonçalves et al. (2006b)

The differences between the groups were larger in the initial than in the final 10-min segments, suggesting that fetal acidemia can already be identified in the first part of the hour preceding delivery and thus a timely intervention is possible (Gonçalves et al., 2006b). This study also suggests that combining both linear and nonlinear indices may provide a better discrimination between normal and acidemic cases.

Fetal Gender

Fetal gender is available nowadays with a high degree of certainty. In the 4 h preceding elective Cesarean section in the absence of labor, male fetuses exhibited a significantly higher linear FHR activity, and a less complex activity than their female counterparts (Bernardes, Gonçalves, Ayres-de-Campos, & Rocha, 2008). This finding suggests that male fetuses express signs of a more active autonomic nervous system and less active complexity control systems.

In the previously mentioned study evaluating fetal acidemia, consideration of fetal gender led to additional findings (Bernardes, Gonçalves, Ayres-de-Campos, & Rocha, 2009). Sixty-six FHR tracings were acquired with internal monitoring from singletons during labor, at 4-Hz followed by 2-Hz resampling. Forty-six fetuses were born with UAB pH $\geq$7.20 (normal group) and 20 fetuses with UAB pH <7.20 (acidemic group). In the minutes preceding delivery, female fetuses expressed higher linear indices, suggesting a greater activation of the autonomic nervous system, while maintaining similar complexity indices (Bernardes et al., 2009). These findings suggest that the two genders exhibit different reactions and adaptation capabilities to stress and distress.

The performance of FHR indices in discrimination between normal and severe intrauterine growth restricted (IUGR) fetuses also was improved when fetal gender was considered (Gonçalves, Bernardes, et al., 2013). In a recent study evaluating 15 severe IUGR fetuses and 18 controls matched for gestational age (Gonçalves, Bernardes, et al., 2013), external FHR monitoring was performed in the antepartum period at a sampling rate of 4-Hz followed by 2-Hz resampling. IUGR fetuses presented greater gender-specific linear and entropy changes, when compared with controls, characterized by a significantly lower entropy and sympathetic-vagal balance in females. Additionally, high sensitivities and specificities were achieved in the detection of IUGR male fetuses, when gender-specific analysis was performed at gestational ages less than 34 weeks (Gonçalves, Bernardes, et al., 2013). Therefore, the inclusion of fetal gender, together with other information such as gestational age, should probably be considered in the diagnosis of fetal distress, supporting the importance of adopting a personalized approach.

Recently, the differences in linear and complex heart rate dynamics were evaluated in twin pairs according to fetal gender combination: male-female (MF), male-male (MM), and female-female (FF) fetuses (Tendais et al., 2014). Fourteen twin pairs (6 MF, 3 MM, and 5 FF) were monitored between 31 and 36.4 weeks of gestation, from which 26 FHR recordings of both twins were simultaneously acquired. Overall, MM twins presented a higher intra-pair average in linear indices than the other pairs, whereas FF twins showed higher sympathetic-vagal balance. MF twins exhibited higher intra-pair averages in entropy indices and MM twins presented lower entropy values than FF twins. MM twin pairs showed higher intra-pair differences in linear heart rate indices than MF and FF twins, whereas FF twins exhibited lower intra-pair differences in entropy indices. The results of this exploratory study suggest that twins have gender-specific differences in linear and nonlinear indices of FHR. MM twins expressed signs of a more active autonomic nervous system and MF twins showed a most active complexity control system. Therefore, fetal gender combination should probably also be considered in the evaluation of FHR variability in twins (Tendais et al., 2014).

Current Developments on External Fetal ECG

One of the obstacles to greater progress in analysis of FHR variability is the limited accuracy of FHR beat-to-beat intervals provided by traditional CTG. External FECG is a possible way to

overcome these limitations, providing noninvasive higher-quality signal acquisition throughout the third trimester of pregnancy (Clifford, Silva, Behar, & Moody, 2014; Silva et al., 2013). These signals may result in better performance of currently used FHR indices, as well as in the development of additional indices.

Recently, under the scope of the Physionet/Computing in Cardiology Challenge 2013 (CinC2013 Challenge) on Noninvasive Fetal ECG, a wide variety of techniques were proposed to extract the fetal QRS (FQRS) from abdominal FECG (Silva et al., 2013). Most of them included the following five-step approach: pre-processing, estimation of maternal component, removal of maternal component, estimation of FHR and RR time series, and post-processing. Among the several proposed approaches, some were based on the wavelet transform (WT). The latter provides a description of the signal in the time-scale domain, allowing the representation of its temporal features at different resolutions (scales) according to their frequency content. For the purpose of locating different waves with typical frequency characteristics, avoiding noise and artifacts, the WT seems a suitable tool for QRS location in abdominal FECG. Although most WT based approaches proposed in the CinC2013 Challenge used WT for de-noising, it was shown that it can also be successfully applied to the FECG detection (Almeida, Gonçalves, Bernardes, & Rocha, 2014), similarly to a previous work of delineating the QRS of the human adult (Martínez, Almeida, Olmos, Rocha, & Laguna, 2004).

An adapted version of the algorithm described by Martínez et al. (2004), focusing on FQRS detection in abdominal FECG recordings, which allows the location of both maternal and fetal QRS complexes, provided a reasonable estimate of the FHR (Almeida et al., 2014). However, its performance strongly depends on the quality of the data, and thus pre-processing methods for discarding very low quality signals need to be considered. The dataset available in the CinC2013 challenge did not include information on the origin of the recordings, and some important factors related with signal quality, such as gestational age, could not be assessed. Nevertheless, the preliminary results suggest that the proposed methodology is able to provide a clinically useful estimation of the FHR. An example of FQRS detection is displayed in Fig. 7.8.

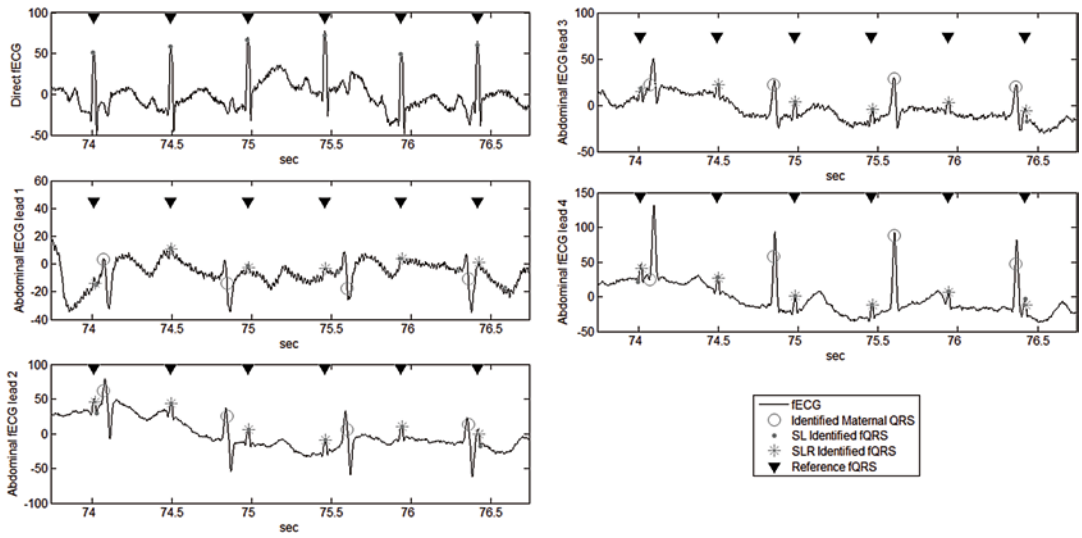

Fig. 7.8 Example of a segment of a direct and a 4-lead abdominal FECG, with the reference and identified FQRS marks. For further details please refer to Almeida et al. (2014)

Concluding Remarks

In this chapter, the importance of incorporating information on fetal gender, gestational age, and behavioral states into computer analysis of FHR variability is pointed out, particularly for prediction of neonatal acidemia and assessment of fetal growth restriction. Additionally, technical aspects of the signal need to be taken into account for the definition of reference intervals in FHR indices, since the mode of acquisition (internal or external) and the sampling/quantization parameters may significantly influence them.

In conclusion, linear and nonlinear analysis of FHR variability are likely to improve the performance of existing computer systems for FHR analysis, justifying more extensive clinical trials with these methods.

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Fetal Auditory Processing: Implications for Language Development?

8

Barbara S. Kisilevsky

Abstract

Fundamental auditory processing abilities such as attention to, discrimination, recognition, and learning of sounds are critical properties of early neurocognitive function, necessary for the acquisition of language, detection of possible opportunity, and identification of impending danger. Over the past 35 years, researchers have characterized auditory processing in human fetuses, occasionally at mid-gestation and reliably from the beginning of the third trimester of pregnancy. Study results demonstrate that fetal gestational age, state of arousal, maternal (e.g., diabetes, hypertension, preeclampsia) and fetal (e.g., growth restriction) high-risk conditions as well as sound frequency, intensity, complexity, and duration influence perception. The finding of differential responding to sounds in fetuses in populations of low- vs. high-risk pregnancies is particularly salient because it has the potential of serving as a marker of neuropathology with one of the most compelling examples the association of atypical response to the mother's voice in growth restricted fetuses and later expressive language deficits. Future research is essential to a better understanding of the underlying mechanisms responsible for disparities in auditory processing, identifying individual fetuses and newborns at greatest risk for subsequent language deficits, and generating and testing novel prenatal and neonatal interventions to prevent or ameliorate communication impairments.

Keywords

Human • Fetus • Audition • Perception • Sounds • Speech • Voice • Language • Heart rate • Body movements

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Introduction

References to human fetal auditory perception occurred anecdotally in the Bible (e.g., Luke 1:39–44) and sporadically in the early scientific literature (e.g., Preyer, 1885/1937; Pieper, 1925; Sontag & Wallace, 1936). However, systematic study has a relatively brief history, beginning in the 1960s–1980s (e.g., Murphy & Smyth, 1962) following the ready availability of ultrasound equipment with sophisticated image processing techniques (see reviews by Kisilevsky & Low, 1998; Lecanuet & Schaal, 1996). Initial work (e.g., Murphy & Smyth, 1962; Dwornicka, Jasienska, Smolarz, & Wawryk, 1964; Bench, 1968) concentrated on auditory abilities in order to learn more about congenital deafness and quickly broadened to examine well-being more generally (e.g., Read & Miller, 1977). This chapter focuses on fetal auditory perception, using selected research primarily from our own laboratory which employed behavioural measures. The chapter begins with a brief theoretical background and overview of the structural development of the auditory system. This is followed by a review of the literature characterizing fetal sensory sensitivity with respect to hearing per se and auditory processing of speech and language. Subsequently, the influence of the uterine environment on fetal response to sounds is explored, particularly the relationship between maternal-fetal heart rate and pregnancy conditions associated with placental insufficiency. It finishes with evidence of a possible link between differential fetal auditory processing in appropriately grown vs. small for gestational age fetuses and later language deficits. Discussion and suggestions for future research are woven throughout. Given the nature of a chapter, there are limitations to what can be covered and such is the case here. The literature review is not exhaustive nor is research from nonhuman species included in a substantive way even though such studies have contributed significantly to our understanding of genetics, fetal physiology, metabolism, and neural development.

Brief Theoretical Background

The developmental origins of health and disease (DOHaD) model attempts to explain the phenomenon by which one genotype can give rise to a range of different phenotypes in response to different environmental conditions during early development (Barker, 2004; Bateson et al., 2004; Gluckman & Hanson, 2004). This epigenetic model of brain plasticity emphasizes the short- and long-term influence of the maternal–fetoplacental relationship. It arose largely from retrospective epidemiological studies demonstrating an association between newborn body weight and the risk for later adult diseases (e.g., hypertension, Barker, Bull, Osmond, & Simmonds, 1990; cardiovascular disease, Barker et al., 1993; diabetes, Hales & Barker, 1992). Based on such work, Barker (1994, 1995, 1997; Barker & Thronburg, 2013; Godfrey & Barker, 1995) put forward the fetal origins of adult disease hypothesis. He posited that fetal growth and development was especially vulnerable to nutrient and oxygen supply during periods of rapid cell division (i.e., critical periods) and that undernutrition at such times permanently changed the fetal body structure, physiology, and metabolism leading to disease in adult life, a phenomenon known as ‘programming’. Different fetal effects resulted in different long-term effects on functional capacity, metabolic competence, and responses to the later environment because the timing of critical periods varied for different tissues with organs becoming sensitive to environmental disparities at different times during organogenesis. The concept of programming underlies the DOHaD model. Over time, the model has been expanded beyond nutrition by demonstrations of other influences during pregnancy on offspring outcome such as maternal stress, both physiological and psychological (e.g., Laplante, Brunet, Schmitz, Ciampi, & King, 2008; King & LePlante, 2015; see Chaps. 12–14), maternal alcohol ingestion (see Chaps. 16 and 17), iron deficiency (see Chap. 15), and exposure to selective serotonin reuptake inhibitors (see Chap. 18).

Recently, Van den Bergh (2011; also, see Chap. 14) purposed the addition of behavior to the model: developmental origins of behaviour, health, and disease (DOBHaD). She argued that integrating early brain and behavioural development in a more elaborate way than the existing DOHaD hypothesis, would allow for a better elucidation of innovative, preventative, and interventional strategies, especially with respect to behavioral problems and psychopathology. The mapping of brain and behavior (and genes and behavior) is complex (e.g., Pennington, Snyder, & Roberts, 2007) and well beyond the scope of this chapter. Simply put, observations of fetal behavior and behavioral change provide a noninvasive method of determining and assessing brain function (Hepper, 1995; Hofer, 1988, 1994). Analyses of fetal behaviours in healthy populations are assumed to reflect functional development of the fetal nervous system during the prenatal period with behaviours emerging and developing continuously over gestation and childhood (Hepper, 2015; Hepper & Shahidullah, 1994). Moreover, perceptual studies in other species have demonstrated that experience (enhanced, species typical, or deprived) can affect the development of sensory systems and behaviour (e.g., Gottlieb, 1971; see Chap. 1). Experimental work in humans is limited. However, spontaneous or sensory elicited behaviors have been shown to differ in fetuses who are neurologically compromised (Horimoto et al., 1993) and as a function of newborn outcome (e.g., Kisilevsky, Hains, & Low, 1999a, 1999b, 2001; see Chap. 16). As such, they have the potential to serve as early markers of neuropathology or pathophysiology. Using behavioural measures, fetal sensory sensitivity has been characterized in both low- and high-risk pregnancy populations, including those associated with placental insufficiency (i.e., undernutrition), demonstrating differential auditory processing between groups as well as an association with early infant language abilities.

Auditory System Development: Concise Overview

The human ear begins development early in the embryonic period (Rubel & Fritzch, 2002) with the neural basis of hearing beginning in the cochlear hair cells (Moore, 2002; Pujol, Lavigne-Rebillard, & Uziel, 1991). By 22 weeks gestational age (GA), the adult number of four rows of outer hair cells and a row of inner hair cells are present on the epithelial surface in the basal cochlea (Pujol et al., 1991) and the cochlea matures over mid-gestation in the absence of auditory input (Rubel & Fritzch, 2002). It should be noted however, that while auditory input may not be necessary, other conditions during pregnancy (e.g., placental insufficiency, Rees, Proske, & Harding, 1989; Rehn et al., 2002) may affect the developing system. Beyond the cochlea, there is a complexity of overlapping layers of cells in the neural pathways leading to the auditory cortex (Cant, 1998) with maturation occurring in a peripheral to central fashion. Myelination of axons occurs at 26–28 weeks GA followed by rapid, synchronous conduction in brainstem pathways at 29 weeks GA (Moore, 2002). At this time, reliable onset of hearing has been observed in healthy, human fetuses (e.g., Kisilevsky, Pang, & Hains, 2000; Shahidullah & Hepper, 1993). The timing also coincides with the onset of reliable otoacoustic emissions (Morlet, Collet, Salle, & Morgon, 1993; Morlet et al., 1995) and auditory brainstem responses in premature infants (Ponton, Moore, & Eggermont, 1996). The predominant mechanism for fetal hearing in a fluid environment is thought to be bone conduction through skull vibration and fluid conduction from the cranial cavity to the inner ear (Sohmer & Freeman, 2001; Sohmer, Perez, Sichel, Priner, & Freeman, 2001). With the onset of hearing, experience with environmental sounds is available for adjustment of the cortical circuits. Moreover, maturation of the auditory system continues with axonal conduction time reaching maturity by 40 weeks GA

(Ponton et al., 1996); path length increases (Moore, Ponton, Eggermont, Wu, & Huang, 1996) and synaptic delays mature into postnatal life (Ponton et al., 1996).

Where in the fetal brain auditory stimuli are being processed is not completely understood. Joseph (2000) argues that responses to short duration, relatively loud white noise are most likely reflexive (i.e., a startle), mediated by the brain stem. But what about longer duration, lower intensity sounds such as speech or music? Results from early imaging studies (Draganova et al., 2005; Hykin et al., 1999) reveal some cortical activity during the third trimester of pregnancy. However, mature axons are present only in the most superficial layer of the cortex and it is thought that it receives little auditory information (Moore, 2002). Another possible site is the inferior colliculus where temporal and spectral aspects of sound are both topographically, but mutually orthogonally, mapped (e.g., Eggermont, 2001). Firm conclusions await future research.

Fetal Auditory Processing: Background

From audio recordings from inside the uterus obtained following delivery, Querleu and Renard (1981), Querleu, Renard, and Crepin (1981), Querleu et al. (1986), Querleu, Renard, Versyp, Paris-Delrue, and Crepin (1988), Querleu, Renard, Boutteville, and Crepin (1989) demonstrated that environmental sounds were available to the fetus from both inside (e.g., maternal heartbeat, bowel sounds) and outside (e.g., voice, music) of the uterus. Individual speech sounds were muffled but the pitch curve of voices was retained absolutely (Querleu et al., 1986). Uterine attenuation was estimated to be about 35–40 dB (e.g., Abrams, Gerhardt, & Griffiths, 1993; Richards, Frentzen, Gerhardt, McCann, & Abrams, 1992) with greater attenuation of high vs. low frequencies (Walker, Grimwade, & Wood, 1971; Querleu et al., 1986).

The fetus is not directly accessible and auditory sensitivity typically has been studied using changes in heart rate and body movements in response to acoustic probes. As noted above, in healthy populations, such behaviors reflect normal central nervous system development (Hepper, 1995; Hepper & Shahidullah, 1994; Hofer, 1988) and provide a noninvasive method of determining and assessing prenatal brain function (e.g., Hofer, 1994; Kok, den Ouden, Verloove-Vanhorick, & Brand, 1998; Low et al., 1992). Fetuses are more responsive in active compared to quiet behavioral states (Schmidt, Boos, Gnirs, Auer, & Schulze, 1985). However, states [quiet (1*F*) and active (2*F*) sleep, quiet (3*F*) and active (4*F*) awake, see Chap. 6 for a detailed description] are not reliably identified electrophysiologically until about 36–38 weeks GA (Nijhuis, Prechtl, Martin, & Bots, 1982) and, even at this late gestation, may be indeterminate or atypical. Furthermore, in some high-risk pregnancy conditions (e.g., maternal diabetes, fetal growth restriction), states may be delayed or disturbed (see Chap. 5). Thus, in order to use the same procedure over gestation and with low- and high-risk pregnancies, as a control for state effects, responding on stimulus vs. sham/silent control trials or periods has been compared to determine stimulus driven behavior.

Fetal Auditory Processing: Hearing

The onset and functional maturation of reliable responding to airborne sound which indicate hearing in healthy, low-risk fetuses was described using a short duration (2.5 s), relatively loud (110 dB), high-pass filtered (800–20,000 Hz) white noise (Kisilevsky et al., 2000). From 29 weeks GA, fetuses responded with heart rate accelerations and body movements. With advancing gestation, the magnitude of the mean cardiac acceleration increased from about 9 to 12 beats per minute (bpm) and the threshold to elicit a response decreased from 110 to 105 dB SPL. At term, our laboratory as well as others have shown that increasing the sound complexity of the stimulus [e.g., pure tone to filtered white noise to

vibroacoustic[1] (mechanical touch + sound); Kisilevsky & Muir, 1991], the frequency (e.g., 500 Hz to 2000 Hz to 5000 Hz; Lecanuet, Granier-Deferre, Cohen, Le Houezec, & Busnel, 1986; Lecanuet, Granier-Deferre, & Busnel, 1988), or the intensity (e.g., 100 dB to 105 dB to 110 dB; Kisilevsky, Muir, & Low, 1989: Lecanuet et al., 1988) will increase the magnitude of the heart rate acceleration. The cardiac response to these brief duration, loud, repeating trains of rapid onset, high frequency sounds which have a response latency of about 4 s, peak at about 12–13 s, and return to baseline at about 20 s (e.g., Kisilevsky et al., 1989) is most likely part of a startle response (Joseph, 2000).

Fetal Auditory Processing: Speech and Language

In order to examine auditory processing with respect to the foundation for language development, more ecologically valid speech stimuli have been presented at longer durations (e.g., 30 s to 3 min) and lower stimulus intensities (e.g., 80–95 dB) so as not to elicit a startle. Collectively, the results of such studies have shown that fetuses discriminate segmented speech sounds and voices over the last trimester of pregnancy. At 36–40 week GA, they discriminated vowel sounds (/i/and /â/; Groome et al., 1999a), the reversal of pairs of consonant–vowel sounds (babi to biba, biba to babi; Lecanuet, Granier-Deferre, & Busnel, 1989) and a change in the gender of a speaker reading a sentence (male to female, female to male; Lecanuet, Granier-Deferre, Jacquet, Capponi, & Ledru, 1993). Younger fetuses, 26–34 weeks GA, also discriminated vowel sounds (/ee/ and /ah/), although higher intensity levels were required to elicit responding (Zimmer et al., 1993). Because these findings can be explained by the acoustic properties of the signal (Joseph, 2000), the influence of experience with such sounds is unclear.

Moreover, research demonstrating maternal voice discrimination (e.g., Kisilevsky et al., 2003) provides convincing evidence that responding is influenced by in utero experience with speech and language. Low-risk, healthy fetuses have been shown to discriminate between their mother's tape-recorded voice and her speaking directly. Without training, audio recordings vs. direct speaking elicited more fetal body movements (Hepper, Scott, & Shahidullah, 1993) and an increase in heart rate (Lee & Kisilevsky, 2014); with 6 weeks of training, fetal heart rate also increased to the audio recordings (Krueger, Cave, & Garvan, 2015). Differential responding in the absence of training might be attributed to a novelty response. Normally, every time that the mother speaks aloud (not whispering), the fetus is exposed to her voice through bone conduction and fluid vibration. Such repeated presentations can lead to habituation. In contrast, an audio recording represents the mother's voice coming from a different place (i.e., outside the uterus) and filtered by the maternal abdominal tissues. While many of the prosodic characteristics of her voice would be retained, intensity, direction, and speech sounds would vary. These resulting changes could represent novelty to the fetus, thereby capturing and renewing fetal attention.

The mother's audio-recorded voice appears to be a particularly salient stimulus for the fetus. In a meta-analysis of laboratory data, we (Kisilevsky & Hains, 2011) found that the onset of a cardiac response to her recorded voice vs. silent control occurred at about 32–34 weeks GA. The initial response was biphasic, a small heart rate decrease followed by an increase. Over gestation the response matured and, by term, the fetus showed only a heart rate increase. Also by term, we (Kisilevsky et al., 2003; Kisilevsky et al., 2009) have shown that fetuses respond differentially to their own mother's voice vs. a female stranger's (previous mother in the study) reading the same story. Across studies, the fetal response has been an increase in heart rate to their mother's voice vs. a

[1] The device used in our laboratory to deliver a vibroacoustic stimulus was an Allied Traders, hand-held, battery powered, cylindrical-shaped (3.5 cm × 25.2 cm) body massager. Frequencies ranged from about 0 to 8000 Hz; the average airborne sound intensity was 75 dB with an average peak of 86 dB A. A lower magnitude of fetal heart rate response and fewer trials to habituate were demonstrated using this vibrator vs. an artificial larynx, indicating a less intense stimulus (Kisilevsky, Fearon, & Muir, 1998).

small decrease, no response, or an offset response to a female stranger's voice. Clearly, to discriminate between the mother's and a female stranger's voice, the fetuses must have had experience with and learned some characteristic of her voice. Given exposure every time that the mother speaks, her voice most likely served as a ubiquitous environmental sound with learning occurring over repeated exposures. The effect is not a generalized effect of hearing a voice because the behavioral effects of hearing the mother speaking directly vs. her audio-recorded voice and the audio-recorded mother's vs. female stranger's voices are different.

These behavioral findings are in keeping with those using functional magnetic resonance imaging (Hykin et al., 1999; Jardri et al., 2012) which reported selective fetal cortical processing for the mother's vs. an unfamiliar voice at 34 weeks GA (Jardri et al., 2012) and fetal cortical activity at term in response to an audio recording of the mother reading a nursery rhyme (Hykin et al., 1999). Taken together these findings indicate attention, discrimination, and some level of cortical processing of the mother's externally presented voice.

It could be hypothesized that fetal recognition of the mother's voice is based on prosodic cues (i.e., the pitch and emphasis contours that give more meaning to speech) as has been suggested for infants (Floccia, Nazzi, & Bertoncini, 2000). However, preliminary analyses of unpublished data in our laboratory suggest an alternate explanation. Mixed model analysis of variance (ANOVA) used to compare heart rate response in 30, 35–37 week GA fetuses to a 2-min audio recording of the mother's voice played backward ($n=20$) vs. forward ($n=10$) showed a cubic effect over time [$F(1, 28)=4.559$, $p=0.04$, partial $\eta^2=0.140$] but no difference between voice direction. As can be seen in Fig. 8.1, the mother's voice played forward and backward elicited a similar pattern of response. Given that manipulation of the voice to its reverse changes the temporal characteristics (prosody), while retaining pitch, frequency, and tone, it would appear that some other characteristic of her voice, or perhaps prosody in combination with some other voice characteristic(s), is being learned during repeated exposure.

Our studies (Kisilevsky et al., 2009) which employed a familiar-novelty paradigm to compare fetal response to the native vs. a foreign language, the mother's vs. female stranger's voice, female stranger's vs. mother's voice, and mother's vs. father's voice (father defined as an adult male cohabiting with the mother during the pregnancy) provide support for the salience of the mother's voice and the conclusion that near-term fetuses have learned some characteristic of her voice as well as their native language. For example, following familiarization with the mother or a female stranger reading in their native language (English), fetuses showed a novelty response to a stranger speaking in a foreign language (Mandarin) but not their native language, replicating previous findings with newborn infants (e.g., Mehler, Bertoncini, Barriere, & Jassik-Gerschenfeld, 1978; Mehler et al., 1988). Following familiarization with either their own mother or a female stranger reading the same passage, fetuses showed a novelty response limited to their own mother's voice, indicating that they recognized the change in speaker from stranger to mother. A novelty response was not observed to a stranger's voice when the voice was changed from mother to stranger, although there was an offset response following termination of the stranger's voice, indicating that the fetuses had heard the voice. When the voice was changed from mother to father, again a novelty response was not observed but an offset response following termination suggested that the fetuses had heard his voice. Subsequently, when response to the mother's vs. father's voice was compared following a week of training with the father speaking to the fetus in his natural voice, a heart rate increase was elicited to both voices, although the magnitude of the heart rate increase to the father's voice appeared to be lower (Lee & Kisilevsky, 2014). Further, after birth, when these same fetuses were tested in a voice preference task, they preferred their mother's voice. Taken together, these findings indicate that repeated exposure prenatally to the maternal voice and the native language sets up some neurological modification that ultimately leads to memories of specific voices and language. Moreover, the structure

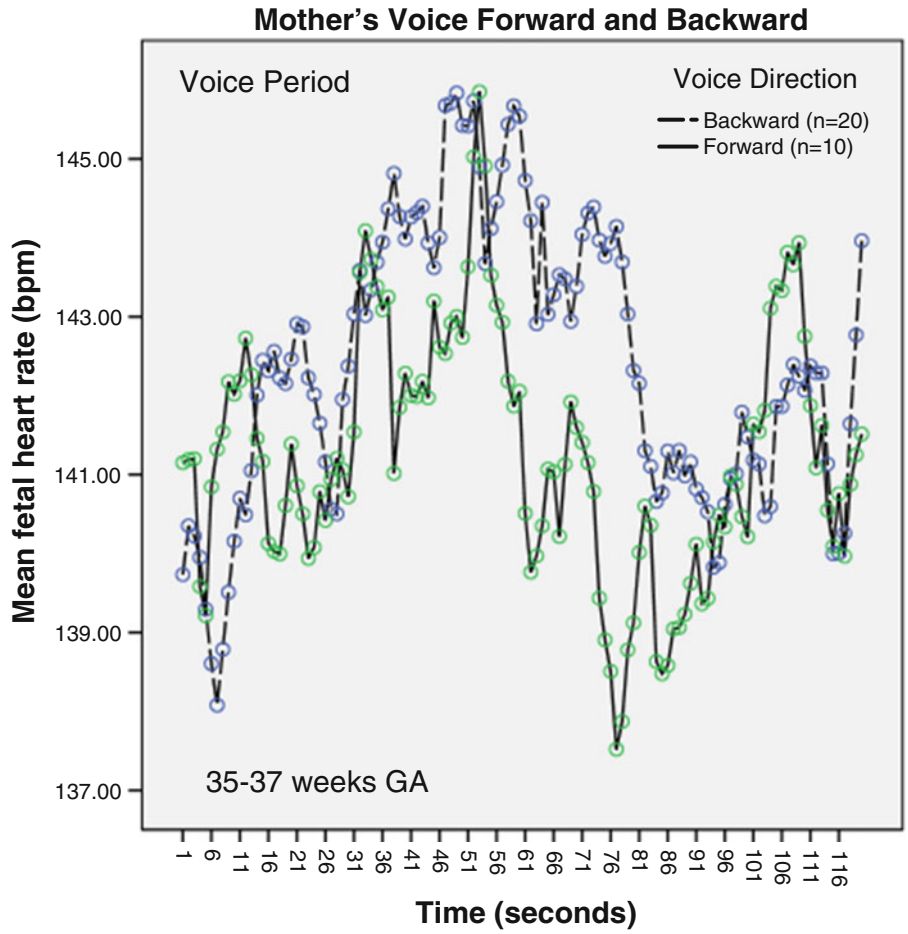

Fig. 8.1 Mean fetal heart rate over 120 s during the playing of the mother's voice forward or backward for fetuses at 35–37 weeks gestational age

and function of the fetal auditory system is not developing in isolation and could be influenced by simultaneously occurring changes in other fetal or maternal biological processes. Our laboratory has examined two possible influences: the relationship between maternal-fetal heart rate and auditory processing in high-risk pregnancies (e.g., threatening preterm delivery, conditions associated with placental insufficiency).

Influence of the Uterine Environment: Maternal–Fetal Heart Rate

While maternal–fetal biological linkages are well characterized and the implications of the symbiotic relationship for fetal development and survival are clear, the nature of the maternal–fetal cardiac relationship has not been as well elucidated. Such an understanding is important when characterizing fetal sensory sensitivity using changes in heart rate measures to ensure that any influence on the fetal response by the maternal system or maternal–fetal system interactions can be taken into account. A brief summary of selected studies is presented here. (For more in-depth coverage see Chaps. 7 and 23) Results of early studies occurring during maternal rest found no relationship between maternal and fetal heart rate measures (e.g., Lewis, Wilson, Ban, & Baumel, 1970) and no reliable periodicities during maternal sleep (e.g., Hoppenbrouwers et al., 1978). More positive results were reported as recording and analysis technologies advanced. In low-risk

pregnancies, a relationship between the maternal and fetal heart rate over 1 and 24 h (e.g., Patrick, Campbell, Carmichael, & Probert, 1982) as well as an association between the maternal parasympathetic indicator (PNS = high frequency/total power) and number of fetal heart rate accelerations (Swansburg, Brown, Hains, Smith, & Kisilevsky, 2005) was reported. Employing fetal cardiotocographic and maternal electrocardiographic measures, no synchrony was observed between maternal and fetal heart rate (DiPietro et al., 2006). Moreover, results of studies using magnetocardiography found occasional beat-to-beat coupling between the fetal and maternal cardiac systems (Van Leeuwen et al., 2003) which could be influenced by controlling maternal breathing rate (Van Leeuwen et al., 2009). Follow-up using surrogate computer modelling, led the researchers to conclude that the synchrony detected was more likely statistical rather than a physiological interaction (Riedl et al., 2009). Examining maternal heart rate variability measures [low frequency power (LF), high frequency power (HF), total power, parasympathetic nervous indicator (PNS = HF/total power), and sympathetic nervous system indicator (SNS = LF/HF)] in our laboratory (Brown, Lee, Hains, & Kisilevsky, 2008), no effects on fetal heart rate were found for any measure in a group of low-risk pairs in normotensive pregnancies. However, fetuses in a hypertensive group whose mothers had a higher PNS indicator were reported to have lower heart rates while the mother was at rest.

To explore associations between maternal and fetal heart rate during rest, data collected simultaneously using the same electrocardiographic equipment (Monica AN24, Monica Healthcare, Nottingham, UK) was extracted (Monica DK version 1.6) from our laboratory low-risk pregnancy database (unpublished) for the preliminary analyses reported below. For 33 pairs, correlational analyses of grouped data revealed no association ($r = 0.03$, $p = 0.85$) between the average maternal and fetal heart rates over 20 min of rest which is in contrast to the positive results reported for longer observation periods (Patrick et al., 1982). Nevertheless, when the pairs were examined individually, the results were mixed (see Table 8.1); significant small positive (18 %) and negative (45 %) correlations (range of r values −0.33 to 0.33) as well as no correlation (36 %) were observed among the pairs.

To explore the influence of changes in one heart rate on the other, fetal heart rate was delayed

Table 8.1 Correlations between average maternal and fetal heart rate in 2-s epochs over 20 continuous minutes while the mother was at rest for each pair separately

Association between maternal and fetal heart rate over 20 min during rest								
Positive correlation			Negative correlation			No correlation		
Pair #	*r*	*p*	Pair #	*r*	*p*	Pair #	*r*	*p*
01	0.13	0.01	04	−0.16	0.000	02	−0.07	0.08
17	0.26	0.000	07	−0.23	0.000	03	0.02	0.62
18	0.15	0.000	09	−0.33	0.000	05	0.06	0.18
38	0.09	0.02	22	−0.14	0.01	08	0.01	0.91
43	0.15	0.000	23	−0.19	0.000	11	−0.02	0.71
50	0.33	0.000	25	−0.11	0.01	26	0.02	0.72
			29	−0.22	0.000	27	−0.04	0.28
			30	−0.17	0.000	28	0.08	0.07
			37	−0.14	0.000	40	0.04	0.37
			39	−0.27	0.000	48	−0.01	0.80
			41	−0.10	0.02	52	0.01	0.77
			42	−0.21	0.000	53	0.04	0.34
			49	−0.27	0.000			
			54	−0.12	0.01			
			56	−0.20	0.000			

by 2 and 4 s with respect to the maternal heart rate and the reverse; none of the delays resulted in a meaningful change in any relationship. Subsequently, to further characterize the relationship, fetal and maternal heart rate variability measures were calculated for the second, 10 min of a 20 min recording while the mother was at rest for 40 pairs using custom software. Fetal heart rate variability measures (LF: 0.08–0.2; HF: 0.4–1.7) were based on David, Hirsch, Karin, Toledo, and Akselrod (2007) and maternal measures (LF: 0.04–0.15; HF: 0.15–0.5) on the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Preliminary analyses revealed significant maternal–fetal associations limited to HF power ($r=-0.37$, $p=0.02$) and PNS indicator ($r=-0.38$, $p=0.01$); both were negative. LF power ($r=0.21$, $p=0.19$), total power ($r=-0.19$, $p=0.25$), and SNS indicator ($r=-0.22$, $p=0.17$) showed no association. Using a median split of the maternal PNS indicator to create two fetal groups, fetal heart rate was examined over the 20 min of rest for the 33 pairs above for whom maternal–fetal heart rate correlations had been calculated. In keeping with our earlier report (Brown et al., 2008), no effect of maternal PNS was observed on spontaneous fetal heart rate change over time in this low-risk sample. Repeating the analyses using a median split of maternal HF, fetal HF and PNS, separately, also showed no effect for any of the heart rate variability measures on fetal heart rate during rest. Collectively, the results over studies using different technologies indicate that, during rest, the maternal–fetal heart rate relationship is complex and may be dependent on the specific heart rate parameter measured/analyzed, the length of observation time, the recording instruments, pregnancy risk status, and whether the data are from an individual pair or aggregated over group. None of the maternal or fetal heart rate variability measures had an effect on fetal heart rate in low-risk, uneventful pregnancies.

Studies of the association between maternal and fetal heart rate measures collected during sensory stimulation are rare. Recently, suppression of maternal heart rate within 5 s of an auditory elicited fetal startle response using a popcorn rattle was demonstrated (DiPietro et al., 2013). Mothers wore headphones with music and eye coverings to mask the sounds being delivered to the fetus. To explore this phenomenon, data were extracted for 20 maternal–fetal pairs for whom a 120-s audio recording of the mother's voice had been delivered to the fetus while the mother wore headphones through which masking music was played. Repeated measures analysis of the mothers' heart rate over 120, 20, 10, and 5 s in the period before vs. during the voice presentation, showed no period, time or interaction effects at either 5 or 20 s before vs. during the playing of the voice (Huynh–Feldt conservative probability reported for all repeated measures analyses). However, over the 10 s before vs. during the fetal stimulus, there was an effect of period [$F(1, 19)=4.786$, $p=0.04$, partial $\eta^2=0.201$] which was linear ($p=0.04$, partial $\eta^2=0.201$). Maternal heart rate decreased an average of 2 bpm over the first, 10 s following the onset of the voice recording.

Over the entire 120 s, there was a time effect [$F(119, 2261)=3.728$, $p=0.000$, partial $\eta^2=0.164$] which was qualified by a period by time interaction [$F(119, 2261)=2.095$, $p=0.02$, partial $\eta^2=0.099$] which was linear ($p=0.04$, partial $\eta^2=0.199$); over both periods, there was a gradual increase in the mothers' heart rate. These results provide a partial replication of the earlier findings. Taken together, the similar observation over laboratories using differing methodologies indicates an initial maternal heart rate decrease following the onset of a fetal auditory stimulus which elicits a fetal heart rate increase and/or motor response. The effect is not likely attributable to the stimulus per se because, in both laboratories, the sounds were masked to the mother. It could be that fetal movement during a popcorn rattle which would elicit a startle influenced the maternal heart rate change, although this is not likely for the maternal voice stimulus as it was below the threshold for a startle.

To further explore the issue, the relationship between maternal and fetal heart rates and heart rate variability parameters were examined using data from these same 20 pairs. As can be seen in Table 8.2, the percentage of pairs showing a

Table 8.2 Relationship between maternal and fetal heart rate before, during, and following presentation of a 120-s audio recording of the mother's voice to the fetus

Voice	Before		During		Following	
Pair #	*r*	*p*	*r*	*p*	*r*	*p*
01	0.11		0.20	0.02	0.09	
02	–0.27	0.01	–0.21	0.02	0.12	
06	0.18	0.04	–0.30	–0.01	0.05	
07	–0.17		–0.03		0.41	0.000
09	0.08		0.46	0.000	–0.10	
11	0.01		0.31	0.01	0.40	0.000
17	0.12		0.54	0.000	0.09	
18	0.08		–0.08		0.24	0.01
20	–0.10		–0.35	0.000	0.02	
22	–0.34	0.000	–0.04		0.65	0.000
23	0.03		–0.26	0.01	–0.17	
25	–0.30	0.01	–0.19	0.04	–0.11	
26	–0.27	0.01	–0.61	0.000	–0.21	0.02
27	–0.02		0.37	0.000	0.08	
28	0.09		0.23	0.01	0.08	
29	–0.12		–0.52	0.000	–0.09	
30	0.41	0.000	0.24	0.01	–0.10	
37	0.03		–0.21	0.02	–0.06	
38	0.10		–0.22	0.01	–0.13	
39	0.13		0.19	0.04	–0.10	

Note: Only significant *p* values are included

significant, small to moderate heart rate relationship during the playing of the mother's voice increased substantially from 30 % before to 85 % during the playing of the mother's voice and then dropped again to 25 % following the offset of her voice.

While this is a small sample and the findings are yet to be replicated, the dramatic increase in relationship between the maternal and fetal heart rates limited to the period of an auditory stimulus played to the fetus (mother was masked) suggests that changes in fetal heart rate or some other physiological process could influence the maternal heart rate. Alternatively, given that 63 % of maternal–fetal pairs showed a relationship (positive or negative) between heart rates over a 20-min period during rest, it could be that fetal stimulation simply demonstrated a relationship more efficiently over 2 vs. 20 min. Only 55 % of pairs common to both analyses illustrated in Tables 8.1 and 8.2 showed a maternal–fetal heart rate relationship both during rest and stimulation which does not provide strong support for the latter interpretation.

An effect of heart rate variability (i.e., HF) on fetal heart rate following the offset of auditory stimulation also has been reported. In an initial study in our laboratory (Smith, Dmochowski, Muir, & Kisilevsky, 2007) employing fetal heart rate obtained by a cardiotocograph, an effect of fetal HF on the fetal cardiac response in the 2 min following the offset of the mother's vs. stranger's voice was found. Using a median split of fetal HF (0.33–0.5 Hz, range restricted due to sample size), the effect was limited to the mother's voice for the group with HF above the median; there was a sustained decrease in heart rate beginning about 40 s after voice offset. To further explore this phenomenon, electrocardiographic data from the 20 pairs noted above were analyzed using a median split of fetal HF (0.4–1.7, David et al., 2007) during as well as following presentation of the mother's voice. Separate analyses during voice presentation and following voice offset revealed an effect of time [$F(119, 2142) = 2.659$, $p = 0.008$, partial $\eta^2 = 0.129$] which was linear [$p = 0.009$, partial $\eta^2 = 0.326$] during the playing of the mother's voice but, unlike Smith et al., there were no significant effects of fetal HF on fetal heart rate either during the playing of the mother's voice or following voice offset. Analyzing the data over the voice and voice offset periods combined showed a time contrast which was quadratic [$F(1, 18) = 4.705$, $p = 0.04$, partial $\eta^2 = 0.207$]. As can be seen in Figure 8.2 which includes the data during and following the voice stimulus, fetal heart rate increased during the mother's voice, the typical response reported at term (e.g., Al-Qahtani, 2005; Kisilevsky & Hains, 2011) and decreased following voice offset for both fetal HF groups. There were no differences in the prevoice period for fetal HF or time and no interactions.

Because there was a relationship between the maternal and fetal HF as well as PNS indicator during rest (see above), further analyses were carried out using a median split of maternal HF, fetal PNS and maternal PNS indicator on the fetal response during and following the playing of the mother's voice. These analyses all showed the

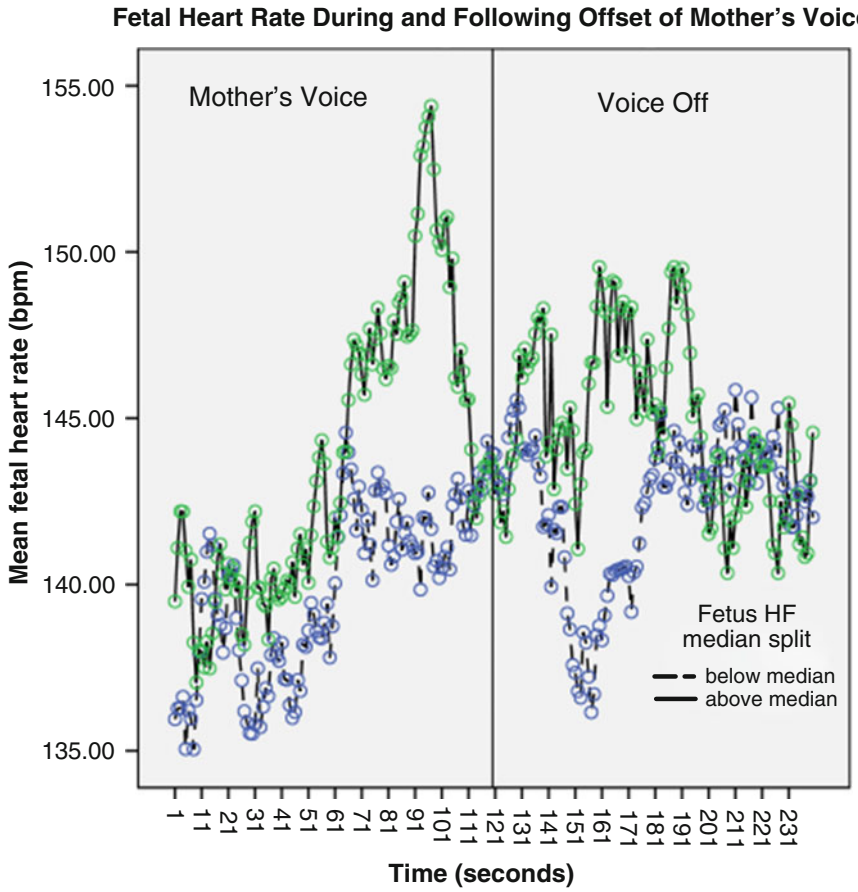

Fig. 8.2 Fetal heart rate during the playing of an audio recording of the mother's voice and following the offset of the recording for those above and below the median fetal HF shown separately

same results as the median split of fetal HF: an effect limited to time. The multiple variations in methodology (e.g., HF range, calculation of median split, recording technology) most likely account for the different findings over studies in our laboratory. Clearly, more research is needed to clarify the maternal–fetal cardiac relationship as well as the influence of sensory stimulation. Presently, fetal HR measures are obtained using diverse technologies (CTG, ECG) with HR variability parameters differing over studies (e.g., HF: 0.3–1.3, Groome et al., 1999; HF: 0.4–1.7, David et al., 2007; HF: 0.50–1.00 Hz, Signorini, Magenes, Cerrutti, & Arduini, 2003). Future standardization would ensure comparability and facilitate replicability of results.

Influence of the Uterine Environment: High-Risk Pregnancies

As well as developing a normative database of fetal perception based on data from those in low-risk pregnancies who delivered as healthy, full-term newborns, our laboratory has been interested in the influence of the 'atypical' uterine environment in high-risk pregnancies which may or may not result in a healthy, short or long-term outcome for the offspring. Positing that differential sensory elicited fetal behaviors in high- vs. low-risk pregnancies would be useful in the assessment of well-being, studies were designed to identify variations between the two populations. Early investigations employed short duration, high intensity

vibroacoustic (see Footnote 1) or acoustic stimuli. In an initial series of studies (Kisilevsky et al., 1999a, 1999b, 2001), we characterized the maturation of cardiac changes and body movement responses elicited by a vibroacoustic stimulus from 24 to 34 week GA, in high-risk fetuses threatening to deliver prematurely (hospitalized, mixed cause) as a function of newborn outcome: 39 % resulted in the term delivery of a healthy newborn; 26 % resulted in a preterm delivery of a healthy newborn; 35 % resulted in a preterm delivery with evidence of compromise (Kisilevsky et al., 1999a). Results showed differences in sensory elicited responding among the three outcome groups as well as when compared to normative data from a group of low-risk fetuses in a previous study (Kisilevsky, Muir, & Low, 1992). The high- vs. low-risk fetuses showed an earlier onset of responding to a vibroacoustic stimulus at 24 vs. 27 weeks GA but a lower magnitude of heart rate increase by 32 weeks GA. Only those high-risk fetuses who went on to deliver as healthy term newborns showed vibroacoustic elicited heart rate and body movement responses by 32 weeks GA that were indistinguishable from those of low-risk fetuses. These studies provided unequivocal evidence of behavioral differences in sensory elicited responding between high- and low-risk fetuses as a function of newborn outcome. The results could not be accounted for on the basis of maturational changes in cardiac or body movement measures occurring simultaneously with sensory development. Neither maturational changes in cardiac variables (i.e., heart rate, number of accelerations ≥15 bpm; Kisilevsky et al., 2001) nor number of ultrasound observed body movements (Kisilevsky et al., 1999b) differed in low- vs. high-risk groups when the membranes were intact. The high-risk fetuses had reduced numbers of body movements only in the presence of ruptured membranes. In a following study, a group of similar high vs. low-risk fetuses were examined using a complex airborne sound, a brief (2.5 s), high-pass filtered white noise (800–20,000 Hz), delivered in air above the maternal abdomen at three relatively loud intensity levels (100, 105, and 110 dB). The onset of hearing was determined to occur at 29 weeks GA, the same as that for low-risk fetuses. Maturation differed, however, with those high-risk fetuses who would be born at term showing an increased magnitude of cardiac acceleration (Kisilevsky et al., 2000).

Subsequently, auditory processing was examined in fetuses in high-risk pregnancies associated with placental insufficiency (e.g., maternal diabetes, hypertension, preeclampsia) vs. those in low-risk pregnancies who delivered as full-term, healthy newborns. While not a homogeneous group (i.e., conditions vary in etiology), the high-risk conditions all have the potential for insufficient oxygen and nutrient provision (undernutrition) for normal fetal growth and development, resulting in fetal/newborn growth restriction. Comparisons of responding to either a vibroacoustic or auditory stimulus also revealed differential responding in these populations. Fetuses in pregnancies complicated by maternal diabetes whether existing prior to or diagnosed during pregnancy (i.e., gestational diabetes; Allen & Kisilevsky, 1999) showed vibroacoustic induced heart rate increases and body movements, indicating that they perceived the stimulus. However, the cardiac response was less mature and less organized and as maternal blood glucose levels increased, fewer body movements were elicited. Fetuses in pregnancies complicated by maternal hypertension responded with a lower magnitude of heart rate increase, fewer body movements, and a lack of cardiac-movement coupling (Warner, Hains, & Kisilevsky, 2002). Moreover, using a brief duration, high-pass filtered white noise, a group of fetuses in pregnancies complicated by preeclampsia exhibited heart rate changes and body movement responses similar to fetuses in low-risk pregnancies (Kisilevsky et al., 2011), indicating that they heard the sound. Taken together, these results provide evidence that fetuses in high-risk pregnancies can hear. Nevertheless, the diversity in response magnitude and maturational changes in responding over gestation in the high-risk groups may indicate deviations in the processing of sounds.

In a series of studies using lower intensity, longer duration recordings of the mother's voice so as not to elicit a startle, again differential responding was evident in the presence of conditions

associated with placental insufficiency vs. low-risk. Fetuses in pregnancies complicated by well controlled gestational diabetes showed no heart rate change to their mother's voice (Kisilevsky, Gilmour, Stutzman, Hains, & Brown, 2012). Similarly, no heart rate change was found for fetuses in pregnancies complicated by maternal hypertension (Lee, Brown, Hains, & Kisilevsky, 2007) or preeclampsia (Kisilevsky et al., 2011). Moreover, in the hypertensive but not the pre-eclamptic group, a heart rate increase occurred following the offset of the mother's voice, indicating that the fetuses had heard the voice. The results of the individual studies were confirmed in meta-analyses conducted on laboratory archival data including fetuses from 23 to 41 weeks GA in high-risk pregnancies ($n=260$) complicated by threatened premature delivery, hypertension or diabetes compared with those in low-risk uneventful pregnancies ($n=233$; Kisilevsky & Hains, 2005). Because behaviors differed not only between the high- and low-risk groups but between those in the threatened preterm labour and hypertension or diabetes groups, it was concluded that the differential fetal behavior could represent adaptation to condition specific insult rather than a generalized response to insult per se. Presently, the mechanism(s) responsible for an effect of those high-risk pregnancy conditions which are associated with placental insufficiency on fetal auditory system functioning is(are) unknown and a matter of speculation.

It could be that dissimilar auditory system development, sensorineural threshold elevation, decreased iron levels and/or thyroid hormone account for the observed differential auditory processing. Diverse auditory system development has been observed in studies of animal models of placental insufficiency. Reductions in axonal diameter that are associated with slower conduction velocities (sheep, Rees et al., 1989) as well as differential brainstem responses indicating delayed myelination and/or changes in synaptic efficacy (guinea pig, Rehn et al., 2002) have been reported. In human fetuses, delayed maturation of auditory evoked responses in growth restricted fetuses was noted based on longer latencies in growth restricted vs. normally grown fetuses studied using magnetoencephalographic recordings from 27 to 39 weeks GA (Kiefer et al., 2008). Thus, it is possible that delays/diversities in system maturation account for the differential auditory responses. Moreover, animal studies showing that delays in myelination and reduction of white matter in fetuses are restored to control levels postnatally (Tolcos et al., 2011) indicate that the effects of auditory system development may be ameliorated following birth when nutrition/oxygenation are no longer compromised.

Alternatively, it could be that there is increased sensorineural threshold elevation because the magnitude of the endocochlear potential is dependent upon oxygen supply (Sohmer & Freeman, 1995). The placenta is less efficient at oxygen diffusion compared to the lungs and, in the presence of placental insufficiency, even less oxygen is being transported to the fetus. Thus, if there were decreased oxygen levels because of placental insufficiency, the intensity of the mother's voice may have been perceived as a less intense stimulus or may not have been loud enough to be consistently perceived. Clearly, fetuses in high-risk pregnancies could hear sounds because they responded to loud sounds, vibroacoustic stimuli, and in some cases, the mother's voice particularly following offset. In future studies, simply raising the intensity of the mother's voice may or may not address the threshold issue as loud sounds can elicit a startle response, confounding processing and reflexive behavior.

Structural and functional deficits in auditory processing have been reported in the presence of lower levels of iron and maternal diabetes, hypertension, and preeclampsia have been shown to compromise fetal iron stores (for a thorough review see Georgieff, 2008 and Chap. 15). Animal models have demonstrated that iron deficiency can have negative effects on myelination (e.g., Connor & Menzies, 1996), structural development of dendrites (e.g., Jorgenson, Wobken, & Georgieff, 2003), synaptic function (e.g., Jorgenson, Sun, O'Connor, & Georgieff, 2005), and brain energy metabolism (e.g., deUngria et al., 2000) which result in abnormalities in hippocampally dependent rodent behaviours (Georgieff, 2008). In keeping with these findings,

infants of diabetic mothers with low iron stores at birth (Siddappa et al., 2004) were reported to show auditory recognition memory deficits which persisted into early childhood (Riggins, Miller, Bauer, Georgieff, & Nelson, 2009). Given the relationship between iron stores and auditory recognition memory, it may be possible to use newborn ferritin levels to determine an effect of iron stores in future studies examining fetal auditory processing.

Level of thyroid hormone also may play a role in differential auditory responses given that cochlear structures are sensitive to the morphogenetic effect of thyroid hormone during the whole duration of maturation (Uziel, 1986). Growth restricted fetuses have been reported (Kilby et al., 1998; Kilby, Gittoes, McCabe, Verhaeg, & Franklyn, 2000) to have reduced circulating levels of free thyroxine (T4), triiodothyronine (T3), and reduced expression of α and β isoforms of thyroid protein. As well as ferritin, it would seem prudent in future studies to measure newborn thyroid levels to examine the effect on auditory processing.

Overall, it may be that disparate auditory system development, sensorineural threshold elevation, decreased iron levels and/or thyroid hormone, individually or in some combination, account for the differential auditory processing observed in the high-risk fetal groups studied. Given that all of these issues can be redressed after birth when increased nutrient and oxygen supplies become adequate for normal growth, environmental sounds are no longer attenuated by the maternal abdomen and tissues, and oxygenation improves with lung diffusion, it is important that future studies determine their role and the short- and long-term persistence of effects.

Fetal Auditory Processing: Influence on Language Development

Given (1) differential fetal response to sounds in high-risk pregnancies associated with placental insufficiency which, in turn, are associated with fetal growth restriction and (2) a 30-year history of follow-up studies reporting an increased risk for language deficits in infants born growth restricted (e.g., 2–5 years—Gutbrod, Wolke, Soehne, Ohrt, & Riegel, 2000; Low et al., 1982; Vohr, Garcia Coll, & Oh, 1988;Walther & Ramaekers, 1982; 5–9 years—Chaudhari, Bhalerao, Chitale, Pandit, & Nene, 1999; Korkman, Liikanen, & Fellman, 1996; 9–11 years—Low et al., 1992), we hypothesized (Kisilevsky & Davies, 2007) that fetal growth restriction affected auditory system development, resulting in atypical auditory information processing in growth restricted vs. appropriately grown fetuses and that speech perception which lays the foundation for later language competence would differ and be associated with later language abilities.

Recently, we reported support for this hypothesis (Kisilevsky, Chambers, Parker, & Davies, 2014). In a longitudinal study, we compared auditory information processing in growth restricted (≤10 percentile, weight for gestational age) vs. appropriately grown fetuses and newborns and assessed neurodevelopment and language in the two groups at 15 months-of-age. As fetuses, the growth restricted group, in contrast to the appropriately grown group, showed a less sustained response to their mother's voice (1 min vs. 4 min in low-risk fetuses). As newborns, again in contrast to the appropriately grown group, they showed no preference for their mother's vs. a female stranger's voice and less recovery to a novel word following habituation. At 15 months, fetuses in the growth restricted group showed expressive language deficits, scoring more than 2 standard deviations below the mean of the appropriately grown group on both the Mullen Scales of Early Learning Expressive Language Subscale and the MacArthur-Bates Communicative Development Inventory Early Word Vocabulary Produced subscale. These results could not be attributed to neurodevelopment in general because, except for the one subscale, similar to the infants who were appropriately grown, the growth restricted infants scored average or above average on all other Mullen subscales (gross motor, fine motor, visual receptive, receptive language). While correlational analyses does not prove causation, the con-

sistency in differential auditory processing observed in a relatively small sample of growth restricted fetuses which persisted into the newborn period and was associated with later expressive language deficits very early in development clearly indicates that this is an avenue of research which is critical to pursue. Language impairments have been associated with numerous clinical disorders that manifest in cognitive (e.g., executive function, Henry, Messer, & Nash, 2012; working memory, Hutchinson, Bavin, Efron, & Sciberras, 2012), social (e.g., autism spectrum disorders, Joshi et al., 2013), emotional (e.g., anxiety disorder, Beitchman et al., 2001), and behavioural (e.g., attention deficit/hyperactivity disorder, Helland, Biringer, Helland, & Heimann, 2012) dysfunction and can have a profound effect on an individual's everyday functioning, academic achievement and occupational status (Johnson, Beitchman, & Brownlie, 2010). Thus, in future studies, it would be important not only to recruit larger sample sizes to begin to identify individual fetuses and newborns at greatest risk for subsequent language deficits but also to begin to test interventions such as systematic presentation of audio recordings of the mother's voice to at-risk fetuses and/or newborns. The latter suggestion is already showing positive results in premature infants (e.g., Krueger, Parker, Chiu, & Theriaque, 2010; Rand & Lahav, 2014) and could have the potential to prevent and/or ameliorate such deficits.

Overall Summary and Conclusions

In summary, this chapter reflects a historical perspective on the study of fetal sensory sensitivity over the past 35+ years. By the start of the third trimester of pregnancy, observations of reliable auditory elicited startle responses (i.e., immediate body movement, increase in heart rate within 5 s) signal fetal hearing and the opportunity for environmental sounds to influence the shaping of neural networks which lay the foundation for later language learning. Subsequently, over gestation, fetuses discriminate speech sounds, voices, and languages. The mother's audio-recorded voice seems to be a particularly salient stimulus. Without training, at term, low-risk fetuses who deliver as healthy, newborns respond during the playing of her voice with a heart rate increase while to other voices, including a female stranger's or the father's, they either do not respond, respond with a heart rate decrease or show an offset response. The mother's voice is a ubiquitous sound to which they would be exposed every time that she speaks. The differential response suggests that repeated exposure sets up some neurological modification that ultimately leads to memory for her voice. Given the importance of the symbiotic relationship for fetal development, the effect on auditory processing of the maternal–fetal heart rate relationship and 'atypical' uterine environments observed in high-risk pregnancy populations were examined. Although relationships were found between maternal and fetal heart rate and heart rate variables, none were found to influence auditory processing in low-risk populations. As well, auditory processing was compared in fetuses in low- and high-risk pregnancy populations. Fetuses in all high-risk groups studied (i.e., threatening premature delivery, diabetes, hypertension, preeclampsia, growth restriction) showed differential responding to a vibroacoustic stimulus, filtered white noise, and/or the mother's voice compared to fetuses in low-risk groups. The reason for the observed differential responding is a matter of speculation at this time. It could be that dissimilar auditory system development, sensorineural threshold elevation, decreased iron levels and/or thyroid hormone between low- and high-risk populations account for the observed differential auditory processing. Importantly, it is possible that the observed differences are predictive of future language abilities. Our short-term follow-up of fetuses identified as growth restricted in utero (confirmed at birth) who demonstrated differential auditory information processing revealed expressive language deficits at 15 months of age. Clearly, future research is necessary to identify individual fetuses and newborns at greatest risk for subsequent language deficits. Testing interventions which have the potential to prevent and/or ameliorate such deficits is imperative as the negative

cognitive, social, and emotional dysfunction associated with communication impairments can have a profound effect on an individual's everyday functioning, academic achievement and occupational status.

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The Fetal Observable Movement System (*FOMS*)

9

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Abstract

In this chapter we describe a newly developed, objective coding system of fetal facial movements. It is argued that such a system is not only necessary to compare results from different laboratories but also has the potential to be used clinically in order to identify compromised fetuses. Furthermore, the system can be used to record fetal behaviors relating to maturation of fetal abilities such as expression of complex facial gestalts as well as sequential movements and reactions to external stimulation (e.g., sound, touch and light).

Introduction

Although developmental changes in the patterns of movement of the human fetus are of interest in medicine, psychology, and philosophy, and a number of researchers have used some of these movements in their research (e.g., Kurjak et al., 2003; Sato et al., 2014; Yan et al., 2006), fetal facial movements are still not fully described (ten Hof et al., 1999). With technical advances of in utero imaging, particularly four-dimensional (4D) imaging (4D ultrasonography), there is now the potential for capturing and analyzing the repertoire of fetal facial movements. Questions concerning the emergence of specific fetal facial movements and how they combine to make recognizable expressions or *gestalts* (De Vries & Hopkins, 2005) still remain. We have developed an anatomically based coding system of muscle movements in the human fetal face called "Fetal Observable Movement System" (*FOMS*), which is a normative coding scheme, based on an analysis of low-risk singleton pregnancies resulting in healthy, full-term neonates. This chapter gives details of this coding system in order to provide a common objective language which permits collection of comparable and replicable data across laboratories.

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Background

Research on facial expressions has a long history. An anatomical account distinguishes between superficial muscles and deep muscles, which by contraction produce a large number of facial displays. According to Fridlund (1994) there are around 20 superficial muscles which, through variation in combinations of contractions, have the potential to produce a great number of expressions. Cohn and Ekman (2005:22) suggest that using these muscles "more than 7000 different combinations of facial muscular actions" can be produced voluntarily using "all permutations of the actions in the forehead area and, for the lower face, all of the possible combinations of two … and three muscles." Superficial muscles are controlled by the seventh cranial nerve. Additionally, the face contains many sensory receptors, including receptors for touch, pain, heat, and cold. Neural control of the lower face is contra lateral with the left side of the brain controlling the right lower side of the face whereas the upper face is both contralaterally and ipsilaterally controlled. Cross-cultural studies suggest that a number of facial expressions, including, happiness, sadness, fear, disgust, anger, and surprise can be recognized across cultures (e.g., Ekman & Friesen, 1971; Fridlund, 1994; Izard, 1971). This is true not only for humans but also animals, such as chimpanzees (e.g., Parr & Waller, 2006).

A number of objective coding systems for human facial movements have been published, notably anatomically based systems such as the *Facial Action Coding System* (FACS: Ekman & Friesen, 1978), the *Album des expressions du visage* (Ermiane & Gergerian, 1978), *BabyFACS* (Oster, 2007), and MAX—the *Maximally Discriminative Facial Movement Coding System* (Izard, 1983). For chimpanzee faces there is a coding system, based on FACS, called *ChimpFACS* (Vick, Waller, Parr, Pasqualini, & Bard, 2007). Such anatomically based systems enable the reliable coding of individual muscle movements of the face. They owe their development to pioneering work by Landis (1924) who reported one of the first anatomically based facial coding systems.

In these anatomically based systems each distinct action of superficial and deep muscles is considered separately and thereby reduces the bias of more impressionistic and subjective interpretations of facial expressions. The anatomically based systems facilitate research on individual muscle movement, rather than the global perception of a facial expression, and have been used to investigate how a specific facial muscle movement is linked to a biological response (e.g., Levenson, Ekman, & Friesen, 1990).

Although a great deal of research on human and nonhuman face has been conducted post birth, relatively little is known about human facial movements in utero. Fetal movements have been described as "spontaneous expressions of an inherently active nervous system" (Hopkins & Reissland, 2010) and could provide an insight into the normal and abnormal development of the nervous system. Less is known about how the development of the fetal face can provide a window into understanding the development of the nervous system. Hence, charting the development of fine motor movements found in the fetal face is particularly important.

Research on fetal facial movements, has described the overall appearance of the fetal face and does not characterize each anatomically distinct movement (e.g., Hata, Kanenishi, Hanaoka, & Tanaka, 2012; Kurjak et al., 2003; Piontelli, 2010).

Such general descriptions have labeled movements using emotionally charged terminology, such as calling a fetal facial expression a "smile" (e.g., Hata, Dai, & Marumo, 2010; Hata et al., 2012; Hata, Kanenishi, Tanaka, Marumo, & Sasaki, 2010; Piontelli, 2010) or "grimace" (Kurjak, Azumendi, Andonotopo, & Salihagic-Kadic, 2007; Kurjak et al., 2003; Kurjak et al., 2005), and thereby implying emotional intent of the fetus. Without anatomically based fine grained scoring however, fetal facial movements are not precisely defined making objective replication problematic. For example, Piontelli (2010:79) suggests that fetal faces before 14–16 weeks appear static and that smiling, anatomically the simplest of all expressions, can be observed occasionally at 15–16 weeks and more

consistently at 18–20 weeks. Anatomically, this "simple" expression can theoretically be based on three different codes in the anatomically based system proposed in this chapter, namely "lip pull," "lip parting," or "lip stretch," which might have different developmental implications. This issue of reliability of an expression was raised by Cohn and Ekman (2005:21) who stated: "Even the smile, which is principally the work of the single *zygomatic major* muscle, typically involves two or three other muscles as well, and not every smile involves the same other muscles."

An anatomically based coding system will permit the coding of fetal facial movements in a specific, objective and reliable manner.

Although over 20 years ago, Fridlund (1994:315) optimistically wrote: "One major shortcoming of past research has been solved. Anecdotal or inductive reportage of human facial displays has been supplanted by anatomically based scoring …." In fact anatomically based scoring is still relatively rare in the majority of fetal research. To date, there are no published anatomically based coding systems that are explicitly designed for fetal faces. Consequently we created the *Fetal Observable Movements System* (*FOMS*), which is to our knowledge the only anatomically based fine-grained coding system which permits the standardized investigation of fetal facial movements.

The Fetal Observable System (FOMS) enables the reliable identification of facial muscle movements of the fetus, focusing on facial movements as well as movements related to the fetal head, such as head rotation and self-touch. A distinct coding system is necessary as the fetal face varies in size and appearance from the newborn, child, and adult face (e.g., Stool, 2001). As the fetal face becomes more mature it begins to resemble the face of a newborn. However, it is not possible to observe or code in the intrauterine environment all the facial movements that can be observed after birth. Facial muscle movements are created by contraction of the growing muscles. In a fetus, maturing facial muscles and innervation of facial muscles result in the increasing ability to produce facial expressions. A number of coding systems have informed the Fetal Observable Movement System (*FOMS*) outlined in this chapter, including FACS (Ekman & Friesen, 1971), BabyFACS (Oster, 2007), ChimpFACS (Vick et al., 2007), and Artnatomy (Flores, 2005).

Development of the Coding System

In the original system, we identified 19 facial movements that occur independently of one another and are visible using 4D ultrasound scans of the fetus (see Reissland, Francis, Mason, & Lincoln, 2011). In the current coding system we include not only facial muscle movements, but also some non-facial codable movements, such as head movements and self-touch. Facial movements (FMs) describe facial actions that can also be observed after birth and form a prenatal to postnatal continuum. Facial touch movements (TMs) are numbered 1–4. Our system is based on investigations of movement patterns of healthy fetuses aged between 23 and 37 weeks of gestation. Given research indicating that fetal facial expressions develop from around 15–16 weeks (Piontelli, 2010), and that innervations of facial muscles develop from around 8 weeks of gestation (Humphrey, 1967), coding fetal facial movements with the anatomically based system could be done on fetuses of younger gestational ages.

In the development of the *FOMS*, fetal faces were observed using 4D ultrasound (Voluson E8) operated by a trained technician and simultaneously recorded directly onto DVDs for off-line analyses. Although it is acknowledged that 4D ultrasound scanning has some limitations, it is considered to be essential in analyzing the facial movements of the fetus in utero (Hata, Dai et al., 2010). Indeed, an increasing number of studies have been reported using this scanning technology to investigate fetal facial movements (Andonotopo & Kurjak, 2006; Hata et al., 2013; Hata et al., 2014; Kurjak et al., 2003; Kurjak et al., 2005; Reissland, Aydin, Francis, & Exley, 2014; Reissland, Francis, Aydin, Mason, & Exley, 2014; Reissland, Francis, Aydin, Mason, & Schaal, 2013; Reissland, Francis, & Mason, 2012; Reissland et al., 2011; Reissland, Mason, Schaal, & Lincoln, 2012; Yan et al., 2006;

Yigiter & Kavak, 2006). The alternative technology, 2D scanning, has been reported in a number of studies, and although it continues to be used for fetal medical scans, such as dating scans, is not suitable for fine-grained facial movement analyses.

Coding Using *FOMS*

FOMS is coded by a trained scorer by reviewing the video-recorded scans frame by frame. Coding is done by systematically viewing areas of the fetal face. Lower face areas are coded first, starting with mouth movements, followed by upper face movements. Reliability is achieved by trained scorers recoding the scans. For every code, start and end times are recorded, as well as the frequency of the occurrence of the number of movements observed.

Data are coded using a computerized recording system for observational data. In our laboratory we use the Observer® system (Noldus, Trienes, Hendriksen, Jansen, & Jansen, 2000). Other software could be suitable. For example, the Multi-Option Observation System for Experimental Studies (MOOSES; Tapp, Wehby, & Ellis, 1995) and the Procoder for Digital Video (PCDV; Tapp & Walden, 1993) can be used in order to enable real time coding and event and time sampling. Similarly, the Behavioural Evaluation Strategies and Taxonomies system (BEST; Sharpe & Koperwas, 2003) could also be used. See Little (2013) for a review of computerized coding systems.

Scan Length and Image Quality

The length of a scan to be coded is dependent on hypotheses to be investigated and the maximum time of scanning allowed. There are restrictions on total scan length from professional bodies. In the UK, total scan length is restricted by the British Medical Ultrasound Society (BMUS) guidelines and further restrictions may be placed by ethical committees. However, as a rule, we have used a 15–20 min total scan length and have coded and analyzed the first 600 s (10 min) of the scan that is codable starting from the first codable movement. We follow British Medical Ultrasound Society (BMUS) guidelines concerning Temperature (TI) as well as Mechanical Indices (TI) (see BMUS, 2009). In our experience normally there are sections of scans which are not codable. For example, there may be sections where the fetal face is entirely obscured by the placenta or not visible because of the position of the fetus. In these circumstances, the coder should suspend coding and restart coding as soon as the face becomes visible again. Furthermore, depending on which area of the face is of interest to an investigation, a scan segment that might be deemed uncodable for one project because part of the face of interest is obscured may be coded for another project where this area is not useful. If the investigator is interested in the eyebrow region of the fetal face, obscured mouth areas might not hinder the coding. If uncodable sections of scan reduce the codable segments of the scan to below the time normally coded this can be taken account of by making adjustments to the statistical analysis.

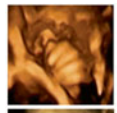
In order to numerically define the confidence with which the scan was coded, each scan is given a quality rating. See Fig. 9.1.

*Scans rated 0 because of obstruction of the face or very poor quality cannot be used for coding specific facial movements.

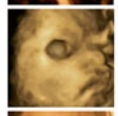
**Scans rated 1 may be used if the gross codable movements are of interest for the research question.

A minimum amount of scanning time is dependent on theoretical considerations. There is no universally agreed time that needs to be used for the analyses. However, there is an upper limit for scans, which are performed following BMUS guides (for scans done in the UK) and which states that the minimum necessary time should be used to scan the fetus and recommending around 15–20 min maximum.

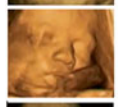
In order to allow comparison between different laboratories it is necessary to record birth outcomes or outcomes at the 20 week anomaly scan. For the purposes of our studies, we recruited fetus considered healthy at the 20-week scan. Future studies might apply the coding system *FOMS* to compromised fetuses.



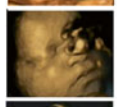
0 - Un-codable vague outlines of fetal facial configuration discernible but un-codable using the FOMS system.



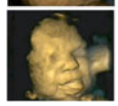
1 - Poor quality allowing the coding of only very gross movements.



2 - Acceptable allowing the coding of parts of the fetal face.



3 - Good with a clear view of the fetal face throughout with few occurrences of un-codable features of the fetal face.



4 - Very Good with a clear view of fetal face with facial features and movements being consistently codable.

Fig. 9.1 Examples of each quality rating

Details of the Fetal Observable Movement System (*FOMS*)

The "Neutral" Fetal Face

The first task of coding using *FOMS* is to observe the neutral face of the fetus. This neutral face helps coders to determine whether a movement has occurred and whether it should be coded. As the fetus matures the appearance of the neutral fetal face will change. Hence, for each fetus at each gestational age the neutral face needs to be reviewed prior to the onset of coding.

Coding Fetal Movements

For ease of cross-referencing, we have used the numbering scheme used in FACS (Ekman & Friesen, 1978) in the *FOMS* coding system. Not all movements coded in FACS can be coded in the fetal face. We use the following terms when describing fetal facial movements. The brow area includes the superciliary ridge above the eyes where the eyebrows can at times be observed in some fetuses and in others this is the area where the eyebrows will grow. A bulge is identified by a protrusion of the skin, where the skin is pushed outward by the muscle, or by skin being stretched over the eyeball or bone. Creasing can be seen when the appearance of the skin is altered and where the skin has folded such that there is a visible line. Although not often seen, there are times when skin covering a nearby area of the face gathers giving the impression of excess of skin in the area. A nasolabial crease gives the appearance of a line or wrinkle, which starts adjacent to the nostril wings and runs down towards the corners of the mouth. Specific fetal facial movements coded in the *FOMS* coding system are detailed below. Facial movements are identified by the acronym FM. The FMs are grouped by the area of the face. See Fig. 9.2 below to aid coding.

FM1: Inner Brow Raise

In a FM1 movement, the frontalis, pars medialis contracts to pull the inner portions of the brows upwards so that the left and right brow areas slope inwards towards each other (see Fig. 9.3). The center of the forehead may show some appearance change but the skin creases seen in adults are only visible in the very young fetus who has not yet accumulated the adipose tissue usually observable in the newborn infants.

Coding specifics: There can be some movement in the outer corners of the brow area as a result of

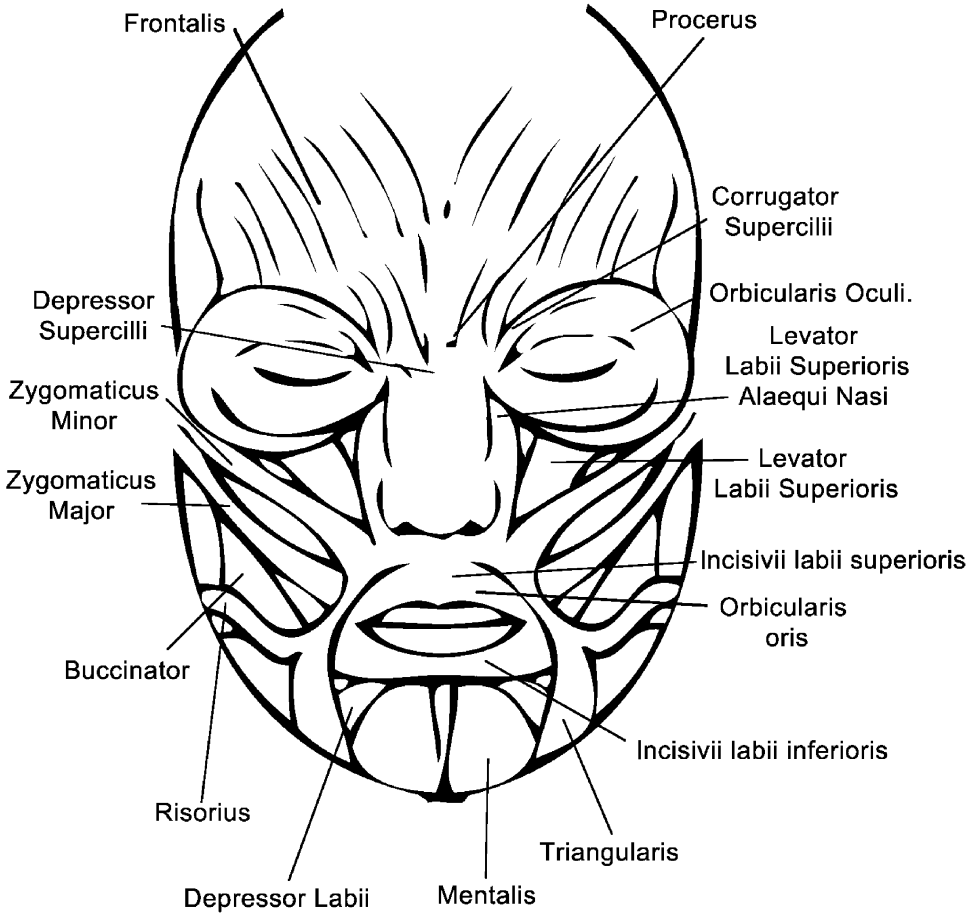


Fig. 9.2 Diagram depicting muscles involved in actions outlined in *FOMS*

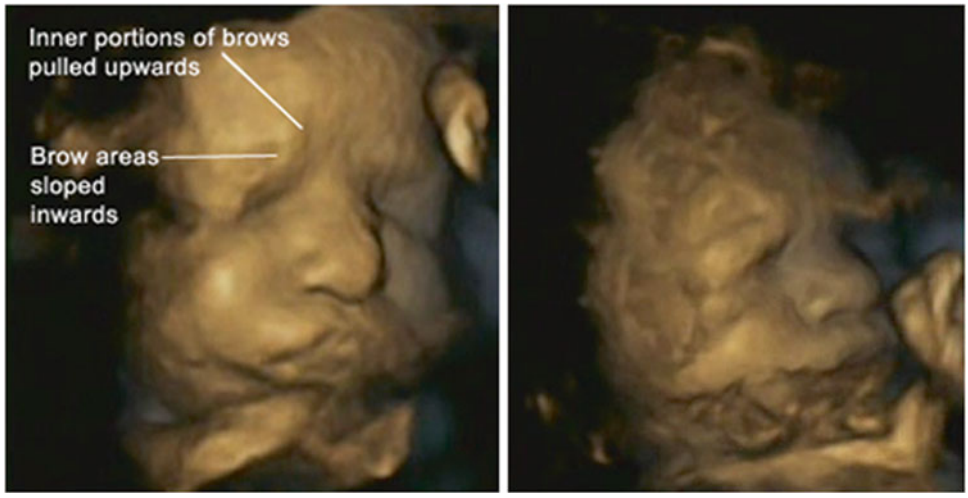


Fig. 9.3 Examples of coding of FM1 including labels of markers of the movement

FM1, as pulling the inner brow areas medially also causes the outer brow areas to be pulled medially. Therefore, FM1 is coded if the brow movement is such that the brow areas together slope inwards towards each other, even if there is some outer brow movement. Both FM1 + FM2 can be coded if it is clear that movements are independent. If the outer brow areas move upwards as well as medially, both FM1 and FM2 may be coded as this demonstrates that the lateral portions of the frontalis muscle are involved in addition to the medial portions.

FM2: Outer Brow Raise

FM2 is caused by the frontalis, pars lateralis contracting to pull the outer portions of the brow areas upwards (see Fig. 9.4). The brow areas appear arched. The brow areas are raised so that the distance between them and the eyes increases. Horizontal creases may appear on the forehead, although these are not always visible, particularly if the scan is taken late in gestation when more adipose tissue has been deposited in the face.

Coding specifics: There can be some movement of the inner corners of the brow area, because pulling the inner brow areas laterally can also pull outer brow areas laterally to a small extent. If the inner brow areas move medially, as well as laterally, both FM1 and FM2 are coded as this demonstrates that the medial portions of the frontalis muscle are involved in addition to the lateral portions.

FM4: Brow Lowerer

In FM4 the brow areas are pulled down by the depressor supercilii muscle and then pulled together by the procerus and the corrugator supercilii (see Fig. 9.5). These muscle movements cause the left and right brow areas to be drawn closer together. As well as the cue of the brow movement itself, FM4 can be recognized by the muscle bulge and skin creases that may appear between the two inner parts of the brows.

Coding specifics: Some markers of FM4 are also markers for other movements. FM6 can cause similar bunching around the eye, especially if the fetus being coded has particularly fleshy cheeks. Coders must ensure that the brow areas are also being pulled down and together for FM4 to be coded. FM6 and FM4 can be coded together, but only if the muscle movements for both are observable.

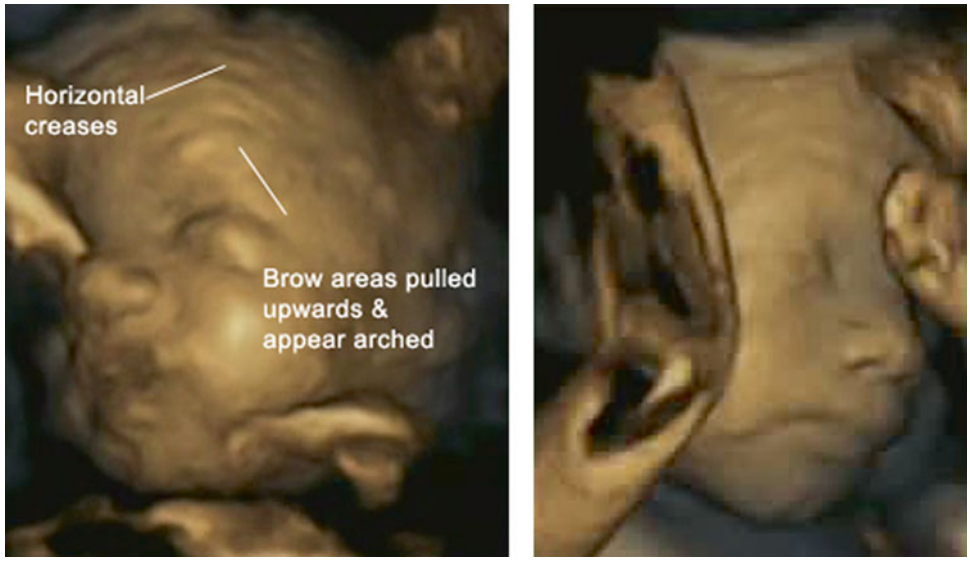


Fig. 9.4 Examples of coding of FM2 including labels of markers of the movement

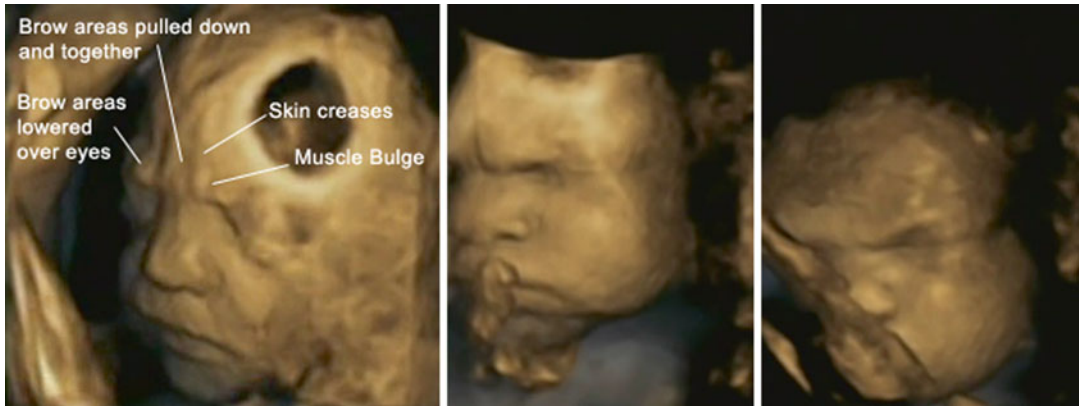


Fig. 9.5 Examples of coding of FM4 including labels of markers of the movement

FM4 can look similar to FM9 as the lowering of the brow areas is a marker for both movements. However, if a nose wrinkle is visible, it must be ensured that the brows are lowering independently of the nose wrinkle and also are being pulled together in order to be able to code FM4.

FM6: Cheek Raise

In FM6 the movement is caused by the action of the orbicularis oculi. Contraction of this muscle causes skin from the cheeks and also from the temple to be pulled towards the eyes. As the cheek is pulled upwards, muscle bulging in the cheek area becomes apparent and skin creases may appear in the outer corners of the eye (see Fig. 9.6). Skin being pulled towards the eye also can cause the eyeball to be slightly obscured from view if the eye is open. Another cue for FM6 is that the nasolabial crease may become evident.

Coding specifics: The upper lip may be raised slightly during FM6. FM10 is not coded with FM6 unless muscle movements are identifiably independent. Some mouth movements may cause the muscle bulge in the cheek that is typical of FM6. FM12 particularly can cause muscle bulges in the cheek area as is typical of FM6. FM6 is only coded if the cause of the movement is the contraction of the orbicularis oculi, demonstrated by skin being pulled towards the eye from both the temple and the cheek.

Lower-Face Movements

FM9: Nose Wrinkle

In FM9 the levator labii superioris alaequi nasi pulls the skin on the side of the nose upwards to where the nose meets the forehead. This causes creases to appear at the top of the nose and may cause the skin around the lower eyelid and from the upper cheek to gather underneath the lower eyelid (see Fig. 9.7). Whereas in adults creases are often also evident along the sides of the nose, these are unlikely to be clearly visible in the fetus, but there will be movement of the skin on the sides of the nose. The nasolabial crease also might become visible, the brow may appear to be lowered and the lower lip may be pulled upwards. Nostrils are likely to show some widening.

Coding specifics: FM9 often involves the brow being lowered but codes FM9 and FM4 are only coded together if it is clear that the movements appear sequentially or if the eyebrows are visibly pulled together. As the crease evident above the nose in FM9 also can be a marker of FM4, this cannot be solely used as an indicator of FM9.

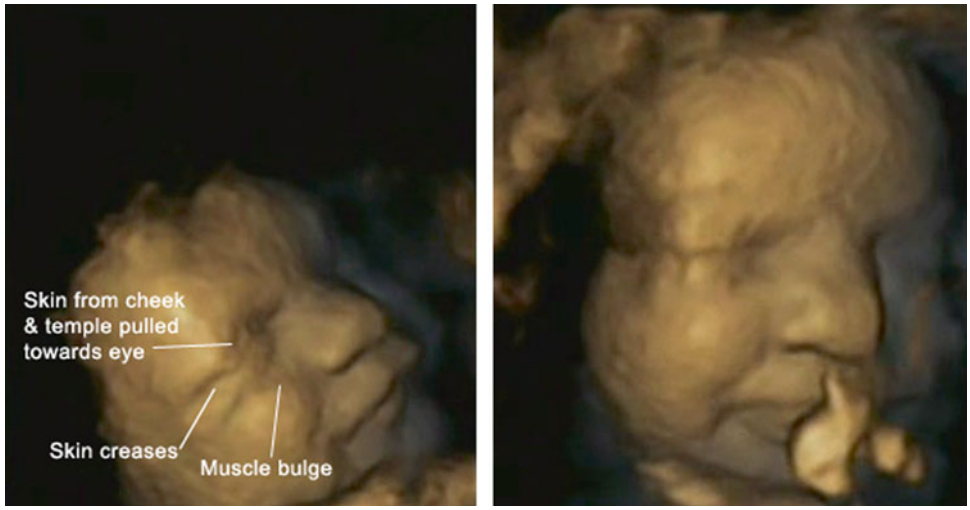


Fig. 9.6 Examples of coding of FM6 including labels of markers of the movement

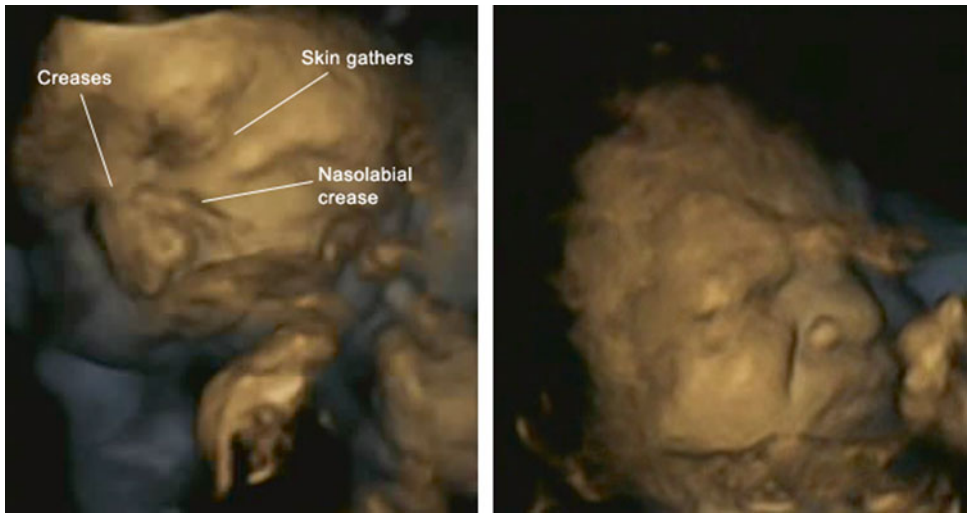


Fig. 9.7 Examples of coding of FM9 including labels of markers of the movement

FM14: Dimpler

FM14 is caused by the buccinator. This causes a small indentation to appear in the cheeks, just above the lip corners. Dimplers are more easily visible later in gestation as they are more prominent in cheeks with more adipose tissue (see Fig. 9.8). There also may be some lateral stretching of the lips. FM14 is more likely to be visible in the older fetus.

Coding specifics: The lateral stretching that can be visible in FM14 can be easily confused with the lateral stretching of FM12. Coders must determine whether it is the buccinator or zygomaticus major muscle that is acting.

FM11: Nasolabial Crease

FM11 is coded when there is a visible deep crease that runs from above the nostrils to the corners of the mouth. This is caused by the action of the zygomaticus minor. The lips may appear to be stretched laterally (see Fig. 9.9).

Coding specifics: There can be some upward, as well as lateral, pulling of the upper lip making it difficult to distinguish FM10 from FM11. In this case, the shape of the crease must be examined. For FM11 the crease runs diagonally from the nostrils to the mouth corners with a small amount of bowing. In FM10, the crease will have an angular bend in it, although this can be only slight, especially when the lip is only slightly raised.

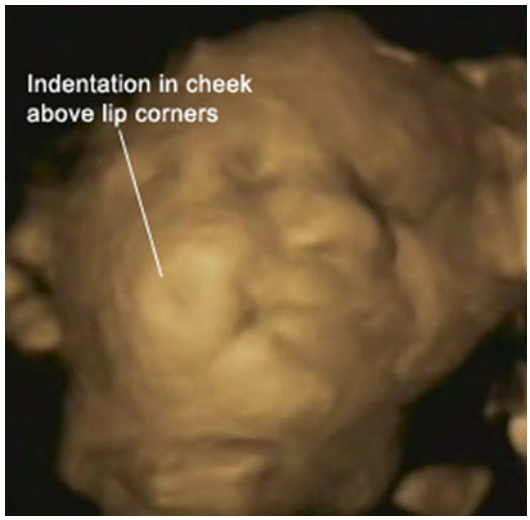


Fig. 9.8 Example of coding of FM14 including labels of markers of the movement

FM17: Chin Raiser

FM17 is characterized by the chin being pushed upwards. This is caused by the action of the mentalis. Contraction of the mentalis also pushes the lower lip up which may cause a depression below the lower lip (see Fig. 9.10). Other visible markers of FM17 are the stretching of skin on the chin and the lip line forming an inverted-U-like shape. The lower lip may also be pushed upwards.

Coding specifics: The inverted-U shape lip line as seen in FM17 is also visible in FM15 where lip corners are pulled down. These two actions must not be confused. FM17 is coded when the action of the mentalis muscle is visible and the chin is visibly pushed up. FM15 is coded if the lips are being pulled outwards and down but not if this action appears to be the result of the chin being pushed upwards.

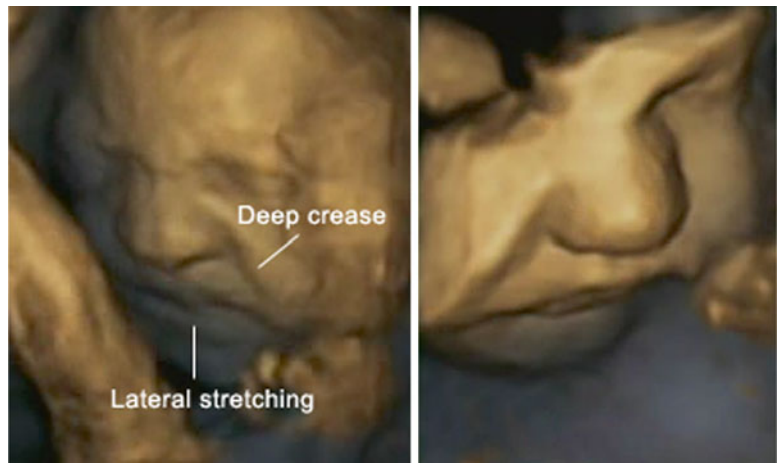


Fig. 9.9 Examples of coding of FM11 including labels of markers of the movement

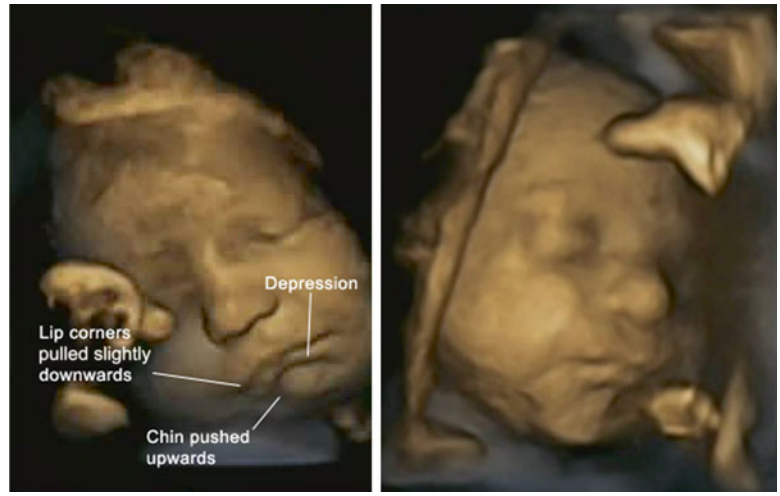


Fig. 9.10 Examples of coding of FM17 including labels of markers of the movement

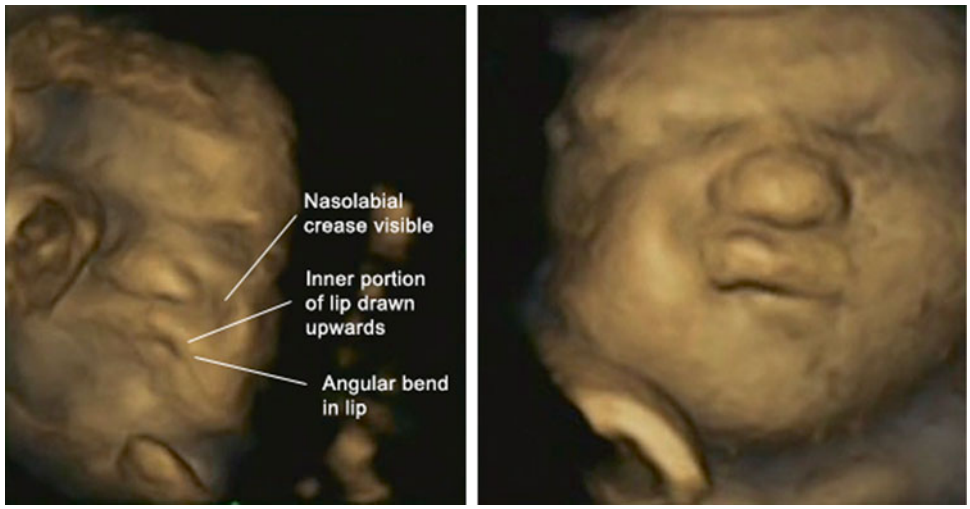


Fig. 9.11 Examples of coding of FM10 including labels of markers of the movement

Mouth Area Movements

FM10: Upper Lip Raiser

FM10 is caused by the levator labii superioris pulling the medial portion of the upper lip upwards towards the nose. The inner portion of the lip is drawn up more than the outer lip to create an angular bend in the lip (see Fig. 9.11). A lip parting can occur and if this is the case, then FM25 should also be coded.

Coding specifics: It can be difficult to distinguish FM10 from FM11. FM10 is coded if the upper lip is being visibly pulled upwards. The shape of the nasolabial crease can also give an indication of whether FM10 or FM11 should be coded. For FM11 the crease runs diagonally from the nostrils to the mouth corners with a small amount of a rounded crease where it meets the nose wing. In FM10, the crease will have an angular bend.

FM24: Lip Presser

In FM24 lips are pressed together so that they appear tight and narrow. This action is produced by the orbicularis oris, pars marginalis muscles in

the lips (see Fig. 9.12). The skin around the lips may bulge because of the lips being pressed together.

Coding specifics: FM24 is often confused with FM18 as both are marked by lips appearing tight. The main distinctions lie in that there is no lip protrusion in FM24 and there is bulging around the lips in FM24 which does not occur with FM18.

FM18: Lip Pucker

In FM18, the lips narrow and purse and the lips will protrude forwards. This is caused by the incisivii labii superioris and incisivii labii inferioris, which pull the corners of the lips medially (see Fig. 9.13). The lips usually appear as if contracted and the mouth opening will look smaller and round. There also may be some bulging on the chin as the skin on the chin is pulled upwards towards

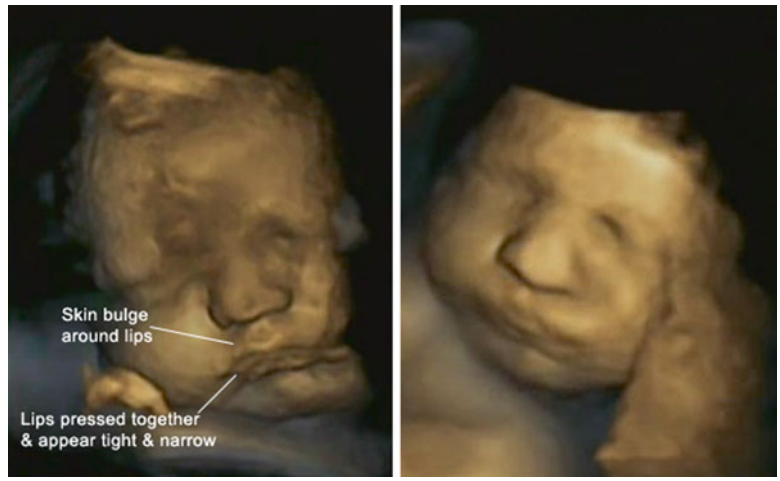


Fig. 9.12 Examples of coding of FM24 including labels of markers of the movement

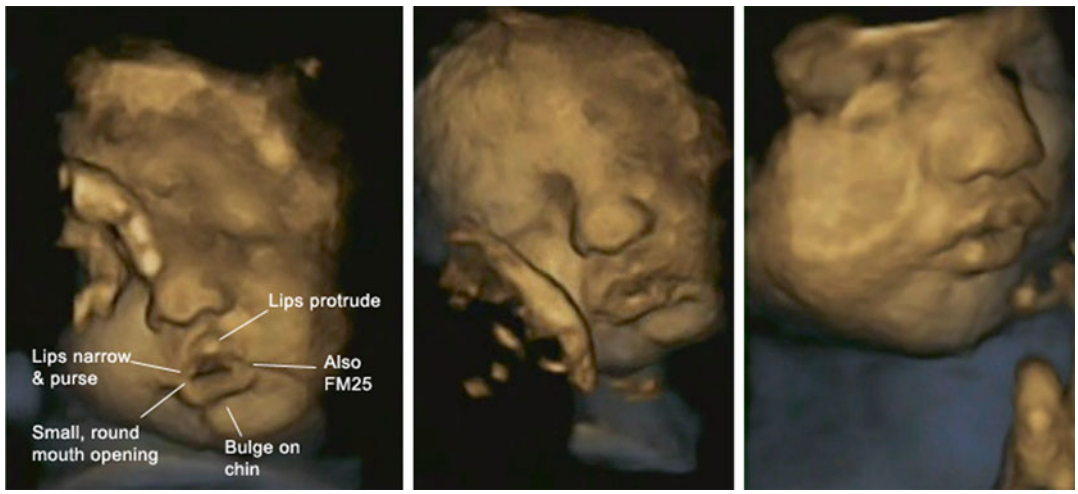


Fig. 9.13 Examples of coding of FM18 including labels of markers of the movement

the lips. It should be noted that FM18 can affect only one lip although usually both are affected.

Coding specifics: In some fetuses, particularly those at a later gestational age, the lips can appear fuller than in others. It is important not to misinterpret this as FM18. This mistake is especially common following a lip action whereby the lips have been stretched such as FM12, FM10, and FM20. FM24 also causes de-elongation of the mouth opening. Thus, it is important not to overcode FM18 by misinterpreting FM24 as FM18. FM25 is coded if the lips are parted in addition to being pursed.

FM12: Lip Pull

FM12 is coded when the corners of the lip are pulled back by the action of the zygomaticus major to create a smile shape whereby the lips are elongated reaching upwards towards the cheeks (see Fig. 9.14). Often there is a bulge visible in the cheeks as the cheeks are pulled upwards towards the eyes.

Coding specifics: The bulge that is often visible in the cheeks when FM12 is involved can sometimes be confused with a movement also produced by FM6. In fetal images, the eyes are mostly closed and skin from the temple gathering around the eye cannot be observed.

FM20: Lip Stretch

In FM20, the lips are stretched laterally by the risorius muscle in such a way that the mouth is elongated. This causes the lips to become stretched and so appear thinner and also causes the cheeks to become stretched laterally. The lips also may move up or down slightly (see Fig. 9.15).

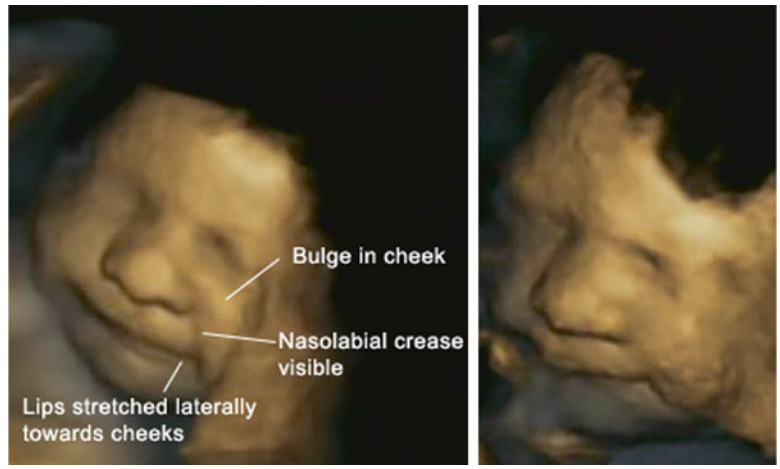


Fig. 9.14 Examples of coding of FM12 including labels of markers of the movement

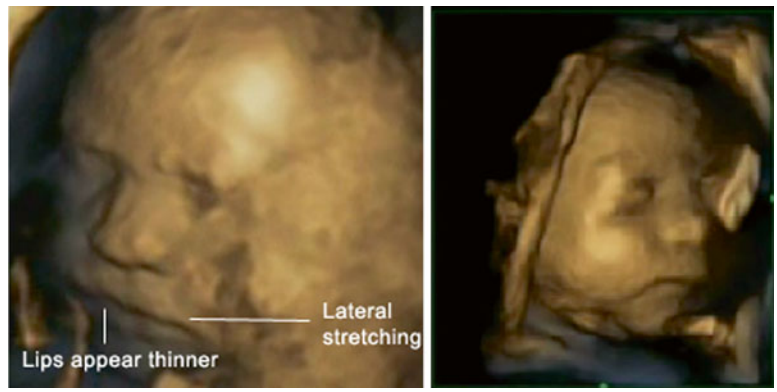


Fig. 9.15 Examples of coding of FM20 including labels of markers of the movement

Coding specifics: As the lips may move down slightly, as well as being stretched, FM20 can be confused with FM15. These actions can be distinguished by examing whether the lip is being pulled down as well as being stretched laterally. Although the lip corners may move up or down slightly in FM20, the main movement is lateral; so if the lip is being pulled down FM15 is coded rather than FM20.

FM28: Lip Suck

In FM28 either the top or bottom lip, or both lips are drawn into the mouth by the action of orbicularis oris, pars marginalis. The lip(s) are then sucked and released. A continuous suck is coded for the duration that the lip(s) are in the mouth (Fig. 9.16). Skin around the lips and the lips appear sucked into the mouth and the skin on the chin will appear stretched.

Coding specifics: None.

FM16: Lower Lip Depressor

In FM16, contraction of the depressor labii muscle causes the lower lip to be stretched laterally and to be pulled downwards. The lip can be caused to either protrude or flatten as a result of this action (see Fig. 9.17). There also may be some appearance change on the chin.

Coding specifics: Often FM16 causes the lips to part. If this happens, lip parting also is coded.

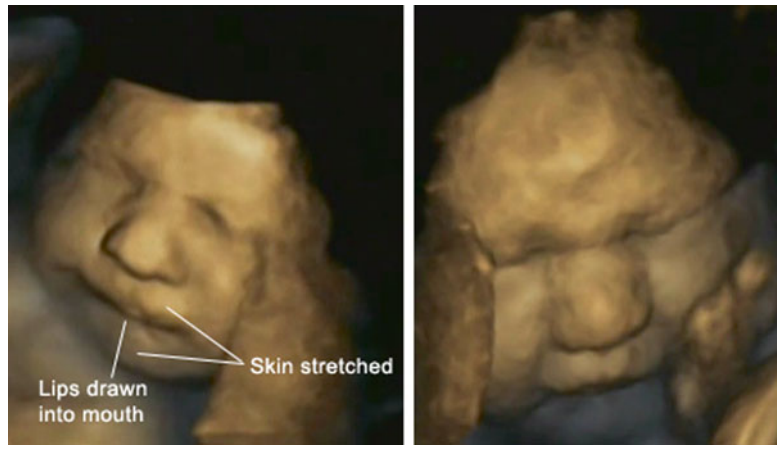


Fig. 9.16 Examples of coding of FM28 including labels of markers of the movement

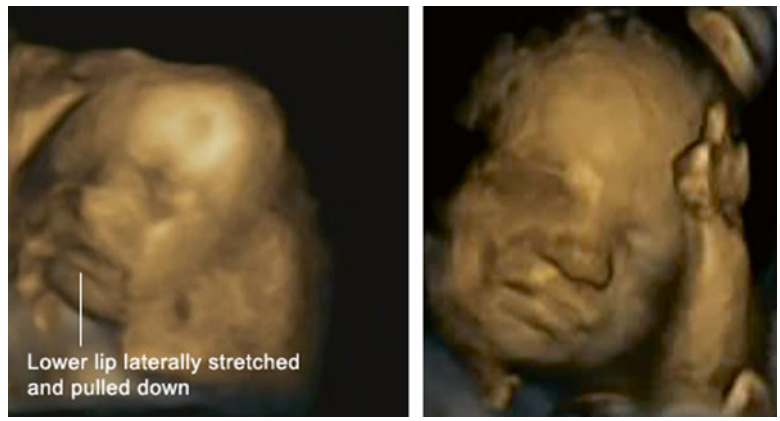


Fig. 9.17 Examples of coding of FM16 including labels of markers of the movement

FM15: Lip Corner Depressor

FM15 is characterized by the corners of the lips being pulled down by the triangularis. There may be distortions of the skin on the chin. Often, there also is creasing of the skin at the corners of the mouth (see Fig. 9.18). The nasolabial crease also may become visible or deepen.

Coding specifics: FM15 is coded only if the lip corners are pulled downwards and FM16 if the lower lip as a whole is pulled downwards. Often, there is some lateral stretching of the lips in FM15 and corners can look slightly down-turned. FM15 can be distinguished from FM20 because in FM15 they are more severely pointed down than in FM20.

FM25: Lip Parting

To code FM25 the lips must part, but the extent to which they part is not specified and can be very slight. The gum area, inner lips and oral cavity may be exposed depending on the extent of the lip parting (see Fig. 9.19). FM25 is caused by the action of the depressor labii muscle although its action is very small or by relaxation of the mentalis.

Coding specifics: If there is a jaw drop, FM27 is coded. FM25 is coded before coding FM27 if there is a lip parting before the jaw drops. If FM27 is a fluid movement, then FM27 alone is coded.

FM27: Mouth Stretch

FM27 is characterized by the lower jaw being pulled down by the action of the external pterygoids and digastricus muscles, so that the mouth is actively opened (see Fig. 9.20). The opening often is stretched such that the longest axis is the vertical plane. The cheeks are stretched and flattened and the skin on the chin also may become bulged.

Coding specifics: It is important to look for an active jaw movement in order to differentiate FM27 from FM25. FM27 is coded when the jaw is being actively pulled down. In the fetus, the lips are usually parted for FM27, but it is possible to see FM27 when the jaw is pulled down but the lips are not parted.

Additional Movements

FM27Y: Yawns

Reissland, Francis, and Mason (2012) found that yawning can be reliably distinguished from simple mouth opening. A yawn is defined by

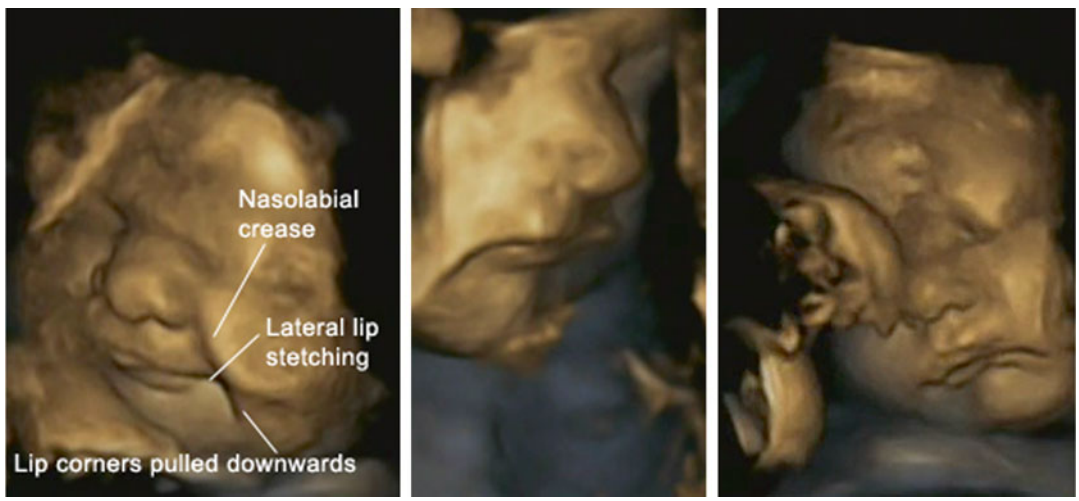


Fig. 9.18 Examples of coding of FM15 including labels of markers of the movement

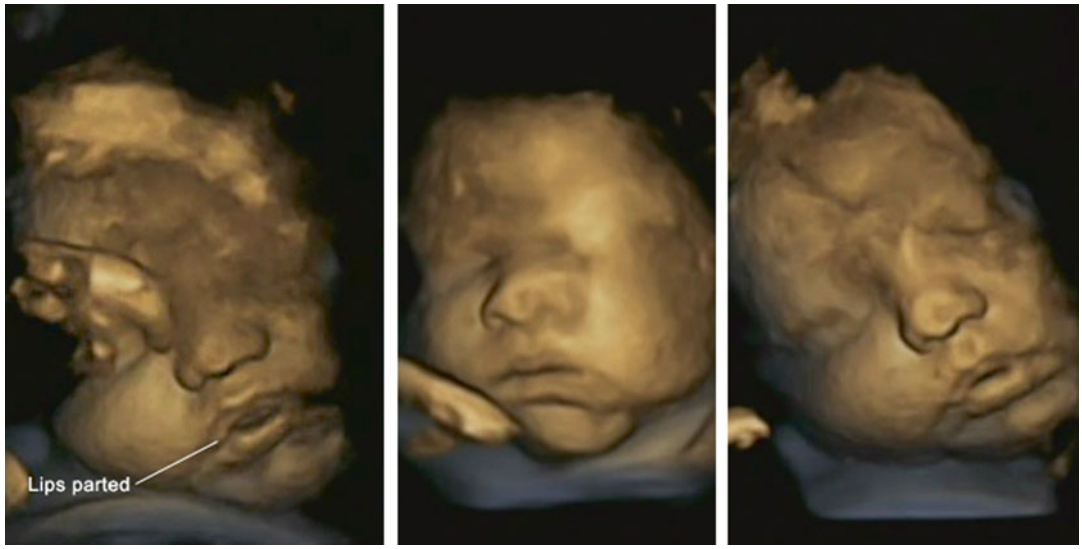


Fig. 9.19 Examples of coding of FM25 including labels of markers of the movement

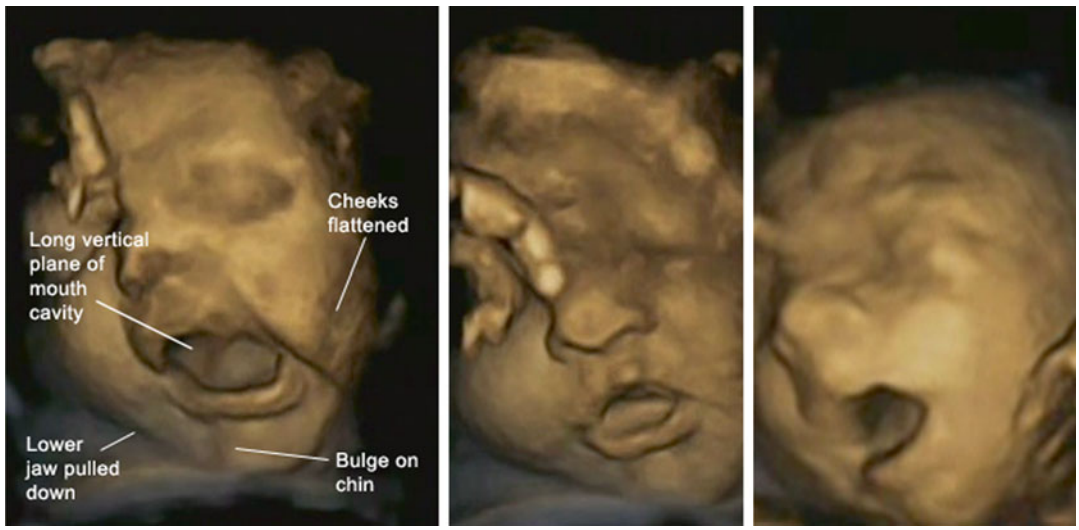


Fig. 9.20 Examples of coding of FM27 including labels of markers of the movement

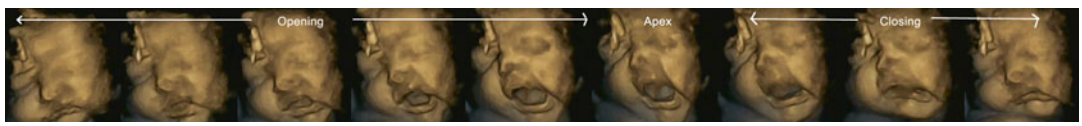


Fig. 9.21 Yawn sequence showing stages of the yawn

Reissland, Francis, and Mason (2012) as a mouth stretch where the length of time between the start of the mouth stretch and the apex of the mouth stretch is more than 50 % of the total time of mouth stretch observed (see Fig. 9.21). This definition corresponds to research, defining yawning as being characterized by a slow opening phase of the mouth and a quicker closing phase (Petrikovsky, Kaplan, & Holsten, 1999). The apex of the mouth stretch is the point at which the

mouth is open widest. At times, there may be a plateau whereby the mouth appears to remain open at its widest point for a while rather than the mouth immediately beginning to close. When this occurs, the apex is determined as the point before there is any closing of the mouth and the plateau is therefore considered to be part of the opening rather than the closing phase. When yawns are of interest, mouth stretches are located and the opening and closing phase times are manually calculated in order to identify each stretch as either a mouth stretch or a yawn.

FM27RJ: Rhythmic Jaw

Rhythmic Jaw movements can be coded if of interest. This has been defined as a sequence of three or more jaw lowering and raising movements (i.e., three or more mouth stretches in sequence).

FM19: Tongue Show

FM19 is a movement during which the tongue becomes visible so that it protrudes beyond the mouth cavity where the tip of the tongue is at least in line with the innermost part of the lip (Fig. 9.22).

Coding specifics: FM19 is not coded when simply being able to observe the tongue in an open mouth cavity. FM19 is coded only if there is some tongue protrusion. If the lips are parted, FM25 or FM27 also are coded depending on whether an active jaw action is visible or not.

EB: Coding of Eye Blinks

Eye blinks are coded from the start of the eye blink movement to the end. The start of an eye blink can be either when the eyes are open or closed. If open, the blink will be coded from the beginning of the eye closing, through to being closed, through to being fully open once more. If initially the eyes are closed, the blink will be coded from the eye beginning to open, through to being fully open, through to being fully closed once more. It is usual for the fetus to predominantly remain with eyes closed. In this case, a blink would be coded from when the eye opens to when the eye closes.

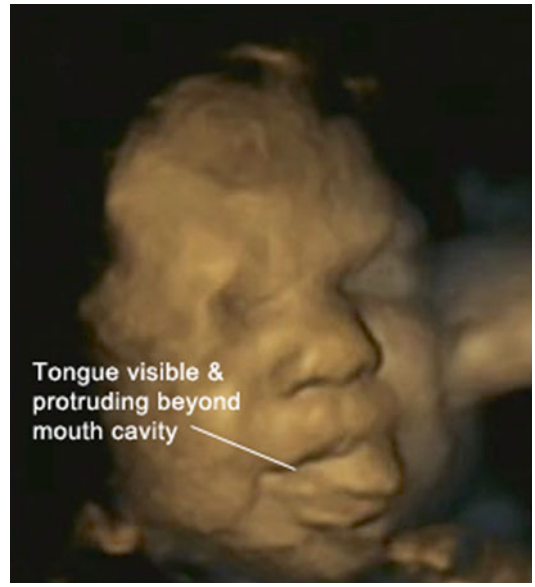


Fig. 9.22 Examples of coding of FM19 including labels of markers of the movement

Coding of Facial Touch Movements

FT1–FT4 Touches to the Face

In order to code the location of limb touches to the face, Reissland et al. (2013) have classified the face into four main areas. These are: touching the upper face area (FT1), touching the side of the face (FT2), touching the lower face area (FT3), and touching the mouth area, which includes touches into the mouth (FT4).

These areas can be seen in Fig. 9.23.

One touch is coded each time contact is made with the face (see Fig. 9.24). Coding begins as soon as contact is made with the face. As soon as the part of the body touching the face is no longer in contact with it, the end of this movement is coded. Such coding enables both the frequency and duration of the movement to be accurately

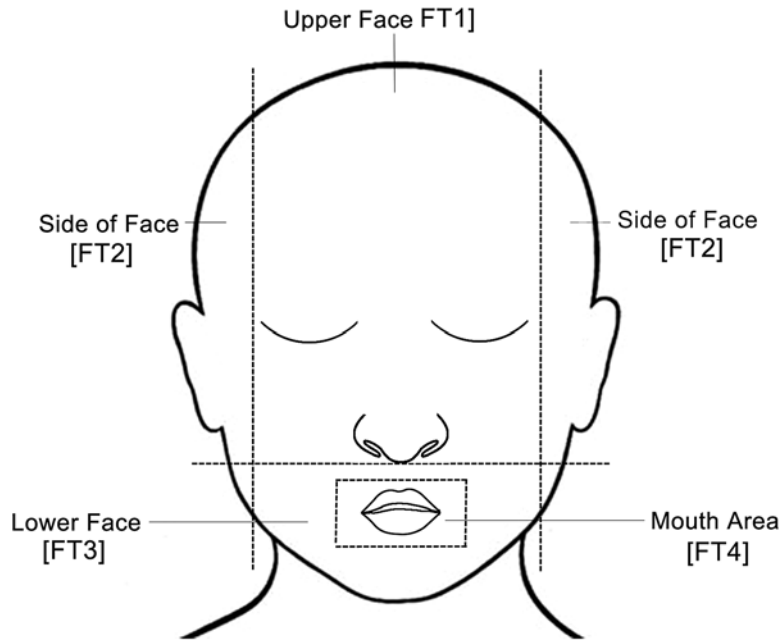


Fig. 9.23 Areas of the face used to code location of touches

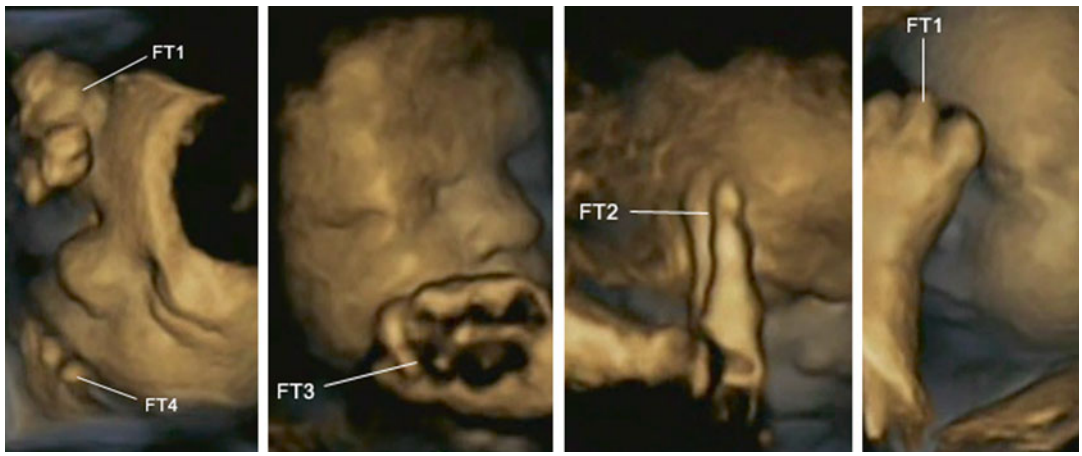


Fig. 9.24 Examples of coding of self-touches (FT1–4) to the fetal face

coded. Laterality of touch may also be coded (i.e., left and right limb touches to the left and right sides of the face).

The angle at which the fetus is visible in the 4D scan can affect the accuracy of distinguishing between when a fetus is touching the face and when it is just near to the face. For this purpose 2D scans can clarify whether there is an actual touch.

Coding of Head Movements

HM1: Head Extension/Flexion

Head extension is coded when there is any form of upward horizontal head movement. Head flexion is coded when there is any form of downward horizontal head movement. These

should be coded from the start of the dynamic movement until the cessation of the movement.

HM2: Head Rotation

Head rotation is coded when there is any form of lateral head movement. This should be coded from the start of the dynamic movement until the cessation of the movement. Head rotations can be subdivided into center-side and side-center movements, and also by left and right movements (from the perspective of the fetus). A fetus may rotate their head slightly, stop the movement for a short period and then again move the head along the same plane. If there is any break in the movement, it is coded as two (or more) separate movements.

Combinations of Movements

Facial Gestalts

In addition to the coding scheme classifying individual observable movements of the fetus, Reissland et al. (2011) developed a method of fetal facial movement coding the muscle configurations that are recognized as emotional expressions. Reissland et al. (2011) considered how the movements observed in the fetus become coordinated over time to form recognisable emotional expressions or "gestalts." They outlined the movements involved in the "cry" and "laughter" facial expressions and later Reissland, Francis, and Mason (2013) described the movements involved in a "pain/distress" gestalt. It should be noted that the expression of these gestalts becomes increasingly complex throughout gestation with more of the individual FMs present simultaneously with gestational age. The muscle configurations of these gestalts are outlined in Table 9.1.

The concept of gestalt coding allows for progression towards a full gestalt. Thus, we can record the number of facial movements which co-occur and which contribute towards a particular gestalt. Figure 9.25 shows an example of a partial "pain/distress" gestalt with four of the six movements present, and

Table 9.1 The 19 fetal facial movements of the coding scheme and the definition of the cry, laughter and pain/distress gestalts based on this scheme indicated by an X

The 19 fetal facial movements	Cry gestalt	Laughter gestalt	Pain/distress gestalt
FM1 inner brow raise	X		
FM2 outer brow raise)			
FM4 brow lowerer	X		X
FM6 cheek raiser	X	X	
FM9 nose wrinkle	X	X	X
FM14 dimpler			
FM11 nasolabial crease	X	X	X
FM17 chin raiser			
FM10 upper lip raiser		X	X
FM24 lip presser			
FM18 lip pucker			
FM12 lip pull		X	
FM20 lip stretch			
FM28 lip suck			
FM16 lower lip depressor	X		
FM15 lip corner depressor			
FM25 lip parting	X	X	X
FM27 mouth stretch	X	X	X
FM19 tongue show		X	

Figure taken from Reissland et al. (2011) and Reissland, Francis and Mason (2013)
Note: for both the laughter and cry gestalts either Lip Parting or Mouth Stretch can occur but not both together

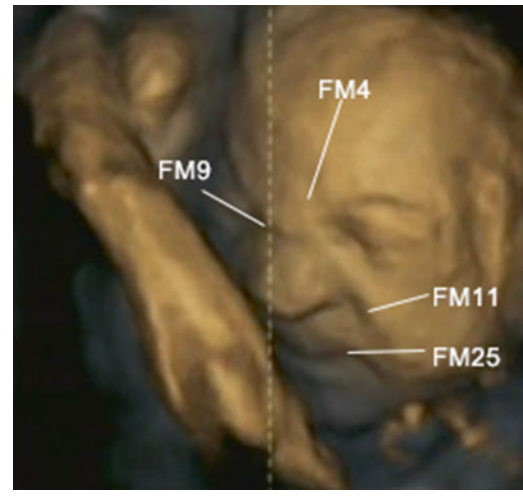


Fig. 9.25 Example of coding of a "pain-face" gestalt

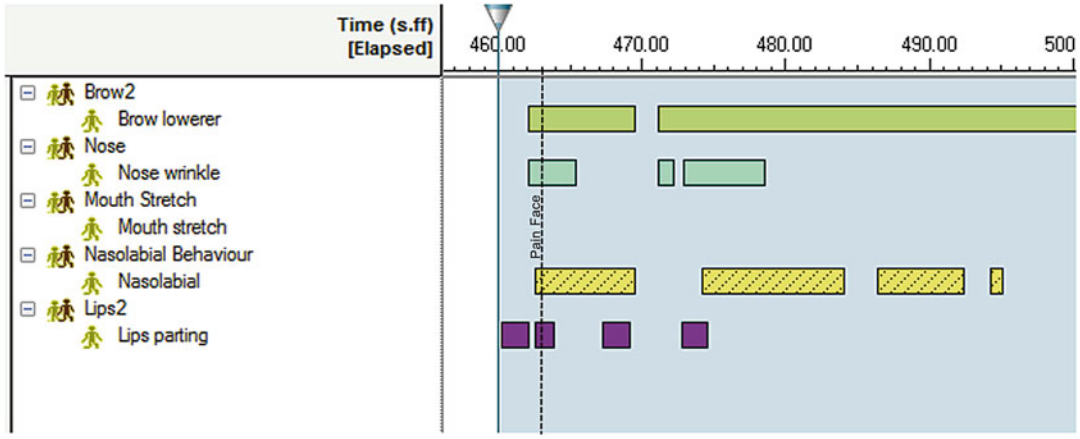


Fig. 9.26 Graph showing the movements shown in Fig. 9.24 coded in the Observer recording system

Fig. 9.26 shows the gestalt movement coded in the Observer coding system.

Related Coding Issues

Reliability of Coding

In order to ensure that the coding of a 4D video-recorded scan is reliable, the scan is coded by two trained *FOMS* coders for each study. We aim to achieve an overall reliability, as assessed by Cohn's Kappa, of over 0.8. When this is not achieved, the scan is recoded by a third coder and discrepancies are discussed so that a consensus may be reached. The overall mean of reliability estimates for research investigating the movements outlined here are 0.91 and the overall mean range is 0.76–0.98.

Limitations of Using 4D Ultrasound Scanning

4D ultrasound scans allow for accurate detection and labelling of movements. This accuracy is demonstrated by high reliability scores being found for the coding of movements. Despite recent advances in the technology involved in producing 4D ultrasound scans, there are limitations to the technique. Scanning is not in real-time with frame-rates used in our studies of 25 frames per second. This means that very fast movements may get missed and movements with a very short duration may not be visible. However, in their review, Hata, Dai et al. (2010) emphasized that 4D scans can be of particular use in the detection of facial movements and expressions in the fetus and suggest that only those movements seen very early in gestation are affected.

Coding Quality of Movements

Reissland & Francis, (2010) have classified the *quality* of fetal movements into jerky or smooth movements. Jerkiness of movements was coded when movements proceeded in a stepwise fashion compared with smooth or fluid movements. The quality of fetal movements has been associated with fetal stress (Reissland & Francis, 2010).

The Effect of Gestational Age on Frequency of Observed Movement

As an example of the use of *FOMS*, we examined the frequency of observed movement for 15 different fetuses observed four times at 24 weeks, 28 weeks, 32 weeks, and 36 weeks. Although most of the fetal scans had 600 seconds of cod-

Table 9.2 Variations in frequency of fetal facial movements coded with the *FOMS* coding system by gestational age ($N = 15$)

Movement code	Frequency rate at 24 weeks	Frequency rate at 28 weeks	Frequency rate at 32 weeks	Frequency rate at 36 weeks	Overall frequency rate
FM1	2.99	3.46	2.80	0.72	2.51
FM2	0.73	0.27	0.00	0.00	0.25
FM4	4.74	8.95	10.63	10.63	8.76
FM6	0.80	1.08	0.70	0.07	0.67
FM9	0.15	0.81	1.47	0.80	0.81
FM10	0.51	0.75	0.98	0.87	0.78
FM11	6.48	10.51	13.50	10.70	10.33
FM12	5.61	6.31	5.80	5.13	5.72
FM14	0.07	0.00	0.00	0.07	0.03
FM15	8.96	14.24	13.22	17.86	13.59
FM16	0.51	0.88	0.84	0.51	0.69
FM17	0.87	2.10	0.21	0.29	0.88
FM18	4.08	3.46	2.59	2.17	3.08
FM19	1.09	1.49	1.05	0.94	1.15
FM20	2.91	1.76	1.47	1.95	2.01
FM24	0.66	0.20	0.14	0.00	0.25
FM25	27.03	29.29	18.39	12.51	21.89
FM27	4.59	2.58	1.96	0.22	2.33
FM28)	1.82	2.24	0.91	1.66	1.66
EB	0.22	3.19	6.50	1.88	2.99

able material, there were a few with less time of the scan being codable. Therefore, the results were standardized to represent the frequency for a 600 second period (or 10 min).

Table 9.2 shows mean frequency rates of healthy fetal facial movements. Without carrying out a formal statistical analysis, we can observe several features. Firstly, some fetal movements have higher frequencies than others. For example FM25 (lips parting) and FM15 (lip corner depressor) occur at a greater rate than all other movements. Some fetal facial movements are rare. For example, FM14 (dimpler) occurs at an overall rate of 0.03 per 10 min of scan and FM2 (outer brow raise) at an overall rate of 0.25 per 10 min of scan. All FMs reported here have been observed. There is evidence that some movements increases with age (e.g., FM4, FM15), and others decline (e.g., FM2, FM24). Secondly, we can observe considerable variability in some of the fetal movements. For example, FM28 (lip suck) varies from a rate of 2.24 at 28 weeks to 0.91 at 32 weeks.

Review of Findings Based on *FOMS*

The coding system of fetal facial movements (*FOMS*) described in this chapter has been used to determine several patterns of fetal development. We were able to show the development of increasingly complex facial movements between 23 and 37 weeks of gestation and combinations of fetal facial movements which become progressively more like the visibly recognizable facial expressions displayed by infants (e.g., the "cry-face" and "laughter-face," Reissland et al., 2011) and the "pain/distress-face," (Reissland, Francis, & Mason, 2013). In line with other research reports (Azumendi & Kurjak, 2003; Mellor, Diesch, Gunn, & Bennet, 2005; Piontelli, 2010; Yan et al., 2006), we suggest that the display of increasingly complex facial expressions is a marker of healthy development. We argue that not only is the developmental transition of movement a process that it likely to be adaptive postnatally (e.g., in promoting bonding and aiding communication) and therefore important to

understand, but also that this understanding has the potential to permit identification of both normal and abnormal developmental pathways.

Although some researchers (Azumendi & Kurjak, 2003; Piontelli, 2010; Yan et al., 2006) have named expressions displayed by the fetus, such as scowling or grimacing, by labeling fetal expressions as they appear when viewing scans, others (e.g., Ekman & Rosenberg, 1997; Fridlund, 1994) have argued that it is necessary to establish a reliable convention of coding where individual movements are precisely catalogued in order to optimize the reliability and usefulness of this line of research. The development of *FOMS* provides an essential tool which can facilitate reliability and reproducibility in future studies.

In addition to demonstrating developmental patterns of movement complexity, we also have shown that the manner in which fetal movement is expressed is affected by fetal stress. Specifically the jerkiness of fetal arm movements, but not fetal leg movements was positively related to fetal stress (Reissland & Francis, 2010). Furthermore, not only an analysis of various movement patterns but also the dynamics of the same mouth movement indicates different behaviors such as simple mouth stretch and yawning which can be traced developmentally. We found that the incidence of both yawning and simple mouth stretch movements decreases at different rates as gestational age increases (Reissland, Francis, & Mason, 2012). Although the function of yawning is unclear, by tracking a developmental pattern of yawning there is the potential for yawning versus mouth stretch to be used as an index of healthy development.

Conclusion

Although a number of laboratories have started to examine fetal facial movements in more detail, there is still a lack of a fine grained and reliable coding system based on anatomical movements which can be observed in 4D scans. With *FOMS*, we believe that data can be reported in a format which allows comparison between groups of fetuses observed in various laboratories and cultures around the world.

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Assessment of the Fetal Neuromotor Development with the New KANET Test

10

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Abstract

Development of ultrasound technology, especially four-dimensional (4D) ultrasound, enabled insight into the fetal neuromotor development that is reflected by the repertoire of fetal activities or fetal behavior. Based on that new technology, the Zagreb group proposed a screening test called the Kurjak Antenatal Neurodevelopmental Test (KANET). Over several years, the KANET has been used to assess almost 2000 fetuses. Results are promising, and the test has demonstrated an ability to recognize normal, borderline, and abnormal behavior in fetuses from normal and pathological pregnancies. However, further studies are necessary as well as long-term postnatal monitoring of children who were prenatally evaluated with the KANET in order to determine its clinical value in identification of children with neurological risk.

Keywords

Fetus • 4D ultrasound • Prenatal neuromotor development • Fetal behavior • Kurjak Antenatal Neurodevelopmental Test

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Introduction

The central nervous system (CNS) develops and begins to mature during the intrauterine period. This maturation is reflected by the repertoire of fetal functions and activities which constantly expands (Marieb, 2001; O'Rahilly & Muller, 1999). Development of ultrasound technology, especially four-dimensional (4D) ultrasound, has enabled insight into the neuromotor development of the fetus and its neurological status. Based on that technology, the Zagreb group proposed a new-screening test KANET, called according to the first author—the Kurjak Antenatal Neurodevelopmental Test (Kurjak et al., 2008). In this chapter we present the most important events of intrauterine neuromotor development, the emergence of the KANET test, and the results of its application.

Prenatal Neuromotor Development

The main events in the fetal neuromotor development are presented in Table 10.1. At 6–7 weeks of gestation, the first synapses in the spinal cord are detectable (Okado, Kakimi, & Kojima, 1979). Establishment of the connections between its motoneurons leads to neuronal activity presented by the earliest movements, called spontaneous vermicular movements (Okado & Kojima, 1984). Moreover, the existence of the first afferent-efferent circuits in the spinal cord is represented by the appearance of the earliest motor reflex activity of the embryo at 7.5 weeks of gestation (Okado, 1981). During intrauterine development, in general, every new form of motor activity is conditioned by the previous development of certain parts of the fetal central nervous system (Salihagic Kadic, Predojevic, & Kurjak, 2009). From 8 to 9 weeks onwards, the first complex, well-organized movement patterns appear as the result of a supraspinal influence on motor activity (de Vries, Visser, & Prechtl, 1982). These movements are called general movements (GM) and they involve the head, trunk and limbs and it is important to emphasize that they emerge in recognizable temporal sequences, without any amorphous movement. This phenomenon can be explained by the intrinsic properties of neurons to generate and propagate action potentials as soon as they interconnect (Stafstrom, Johnston, Wehner, & Sheppard, 1980).

From 10 weeks onwards, fetal movements become more numerous and frequent (Salihagic Kadic et al., 2009). This growth in frequency and number (quantity) of fetal movements comes as a result of the medulla oblongata maturation ahead of other structures of the brain stem. Consequently, until the end of gestational weeks 10–11, breathing-like movements, alterations of the heart rate and reflexive movements of the head, body, and extremities appear prior to other functions (see Table 1, Joseph, 1999). Facial movements emerge around 10–11 weeks (Mulder, Visser, Bekedan, & Prechtl, 1987). At the same time, the fetus begins to show the earliest signs of right- or left-handedness which might be stimulated from fetal motor activity as a result of its influence on brain organization (Hepper, McCartney, & Shannon, 1998; McCartney & Hepper, 1999). From gestational week 13 onwards, a target point of each hand movement and their "goal-orientation" can be recognized (Kurjak et al., 2003). Finally, at 13–14 weeks, isolated finger movements can be observed (Pooh & Ogura, 2004).

Our longitudinal study, performed by 4D ultrasound in 100 fetuses from all trimesters of normal pregnancies, has shown stagnation of the startle movement pattern in the first trimester (Kurjak et al., 2006). However, it has shown increased frequency of GM, isolated arm and leg movements, stretching and head movements (Kurjak et al., 2006).

In the second trimester, the range and complexity of the fetal behavioral patterns expands. Between 15 and 17 weeks of gestation, development of the subplate zone begins which is a zone for transient synapses and neuronal connections (Kostovic & Rakic, 1990). In the second half of pregnancy, the gradually organized fetal movement patterns followed by the rest–activity cycles become recognizable (D'Elia, Pighetti, Moccia, & Santangelo, 2001; Natale, Nasello-Paterson, & Turlink, 1985). At 28 weeks, isolated eye-blinking patterns become one of the most frequent facial movements as the result of the

Table 10.1 Summarized neuromotor development in utero [adapted with permission from Salihagic et al. (2009)]

Development of the fetal nervous system	Fetal motility
First trimester—early stage	
Spinal cord—first synapses (6–7 weeks)	Slow flexion/extension of fetal trunk at 7–7.5 weeks (vermicular movements)
Brain stem (7 weeks)	First motor reflexes at 7.5 weeks
Basic structures of the diencephalon and cerebral hemispheres (8 weeks)	General movements(GM) at 8–9 weeks (head, trunk, and limb movements)
First trimester—late stage	
Brain stem structures maturation (primarily medulla oblongata—VIII–XII cranial nerves	Isolated limb movements at 9 weeks
Pons (V–VIII cranial nerves)	At 10 weeks breathing-like movements
	At 10 weeks head flexion and rotation
Delayed maturation of the midbrain	Yawning at 10–11 weeks
First synapses in the cerebral cortex (end of the 10 week)	Handedness at 10 weeks
	At 13 weeks goal orientation
Second trimester	
Brain stem maturation continues	At 14–19 weeks period of high fetal activity
Almost completely mature medulla oblongata by the end of this trimester	Appearance of eye movements at 16–18 weeks
Formed subplate zone (15–17 weeks)	Fetal motor patterns start to organize at 20 weeks (rest–activity cycles)
Most intensive formation of the synapses (15–20 weeks)	At the end of the second trimester peak frequency of facial movement patterns (except eye blinking)
First electrocortical activity (19 weeks)	
Appearance of the spinothalamic tract (20 weeks)	
Formed thalamocortical connections (24–26 weeks)	
Third trimester	
Brain stem maturation continues	Peak frequency of the eye-blinking pattern at 28 weeks
Spinothalamic tract begins to myelinization at 29 weeks	Facial expression patterns start to decrease or stagnate
Evoked potentials from the cerebral cortex (26–28 weeks)	Number of GMs decreases and complexity increases
Cortical area begins to differentiate (24–34 weeks)	At 33–38 weeks increase in complexity of eye movements
Start of the lamination neocortex from 32 onwards	At 36–38 weeks behavioral states begin to establish

maturation of the mesencephalon in the second trimester with grimacing, sucking, and swallowing (see Table 1, Kurjak et al., 2006). Eye-blinking pattern appears around 18 weeks of gestation, and then the frequency gradually increases. Recently, it has been shown that retinal amacrine cells are sensitive to motion and also responsible for motion vision and that ocular movement predicts postnatal eye function (e.g., motion vision) of the newborn. These movements also represent an important indicator of fetal health (Baguma-Nibasheka, Reddy, Abbas-Butt, & Kablar, 2006).

In the third trimester, the cerebral cortex is still very immature, though six-layered lamination appears as a result of neuronal differentiation and the laminar distribution of the thalamocortical axons (Kostovic, Judas, Rados, & Hrabac,

2002). The brain stem, which is in the maturation process, remains the main regulator of the fetal movement patterns (Joseph, 1999). This is a result of the earlier onset of maturation of the lower motor control system prior to the upper one. The upper motor control system, consisting of the cerebral hemispheres and basal ganglia emerges at 34 weeks of gestation (Amiel-Tison & Gosselin, 2009). However, functional connections between the periphery and cortex operate from 26 to 28 weeks onwards. The connection of the periphery and the central nervous system was indicated by evoked potentials that can be registered from the cortex (Klimach & Cooke, 1988).

During the last weeks of pregnancy, it is possible to follow the organized behavioral states as the eye movements become integrated with heart rate and fetal movements. Moreover, GM patterns start to change in the last 10 weeks of pregnancy; their number decreases and complexity increases as the result of maturation of the brain stem (Merz & Weller, 2005; Pomeroy & Volpe, 1992; Salihagic Kadic et al., 2009). The frequency of the variety of facial movement patterns, such as mouthing, yawning, swallowing, or grimacing, also decreases or becomes stagnant in the third trimester (Kurjak et al., 2006).

Furthermore, in our studies, we demonstrated that there were no movements observed in fetal life that were not present in neonatal life. Hence, prenatal–neonatal continuity exists even in subtle, fine movements such as facial mimics (Kurjak et al., 2004; Stanojevic et al., 2011).

Prenatal Neuromotor Assessment Using the KANET

Numerous studies employing conventional two-dimensional (2D) ultrasound have shown that normally developing fetuses and fetuses at risk exhibit different patterns of behavior. This led to the conclusion that fetal behavioral patterns directly reflect processes of development and maturation of the fetal central nervous system (de Vries & Fong, 2006, 2007; Rosier-van Dunne, van Wezel-Meijel, Bakker, Odendaal, & de Vries, 2010). It also led to the conclusion that the assessment of fetal neurobehavior may make it possible to distinguish normal from abnormal brain development as well as early diagnosis of various structural and functional abnormalities (Prechtl, 1990). However, 2D ultrasound was considered a somewhat subjective method because the information needs an observer interpretation. Some of these limitations of 2D methods have been overcome with the latest development of three dimensional (3D) and 4D sonography (Andonotopo et al., 2005). These later developed technologies enabled precise study of fetal and even embryonic activity and behavior. While 3D ultrasound freezes the image of an object and, therefore, does not provide any information on movements, 4D ultrasound enables the opportunity of simultaneous visualization of the movements of the head, body and all four limbs and extremities in three dimensions, in real time. Using 4D ultrasound in obstetrics, it is possible for the first time to monitor quality (complexity and variability) and quantity of fetal movements on 3D real-time reconstructed images (Kurjak, Miskovic, et al., 2007). With this new diagnostic tool, it is possible to see a greater quantity of movements compared with using 2D ultrasonography (Andonotopo et al., 2005). 4D ultrasound gives a better estimation of movements in general. Also, we can discover subtle ones like rotations and changes in direction of movements (Andonotopo et al., 2005; Kurjak, Miskovic, et al., 2007; Lee, 2001). The above features are especially important in the assessment of general movements. Using experience in 4D sonography and knowledge in the neuropediatric field, the Zagreb group proposed a new prenatal screening test for the assessment of fetal motor activity and it was named after the first author, the Kurjak Antenatal Neurodevelopmental Test (KANET) (Kurjak et al., 2008). It involves some elements from the postnatal test, The Amiel-Tison Neurological Assessment at Term (ATNAT) and GM assessments (Amiel-Tison, 1990, 2002; Amiel-Tison & Gosselin, 2009; Amiel-Tison, Gosselin, & Kurjak, 2006; de Vries et al., 1982; Hadders-Algra, 2004; Kurjak et al., 2008; Prechtl, 1990).

According to Amiel-Tison, the presence of three signs offers a precise clue to fetal brain

damage. Since two of three signs can be seen in utero, they also are included in the KANET (Amiel-Tison, 1990; Amiel-Tison, 2002; Amiel-Tison, Gosselin, & Kurjak, 2006; Amiel-Tison & Gosselin, 2009; Amiel-Tison, Gosselin, & Gahagan, 2005; Gosselin, Gahagan, & Amiel-Tison, 2005). These three signs are: high-arched palate (due to insufficient molding forces of a hypoactive tongue), nonreducible adduction of the thumb in a clenched fist (due to absence of spontaneous motor activity) (see Fig. 10.1), and cranial ridges over each suture or restricted to the squamous suture (due to severe or moderate impairment of hemispheric growth). As for now, the visualization of high-arched palate routinely with 3D surface imaging remains impossible because the technique does not permit simple visualization of deep structures in the oral cavity.

Further, clinical studies have shown that the quantity of GM is a poor indicator of fetal well-being because of the great intraindividual and interindividual differences and great overlap between the normal and abnormal. In contrast, changes in elegance and fluency as well as fluctuating of intensity and speed of GM (quality) were shown to be prominent in sick preterm infants (Prechtl, 1990). In the Gestalt-perception, GM are judged as abnormal if they are monotonous, if they are repetitive in pattern, have less complexity or if they are "cramped-synchronized." GM are finally classified according to Hadders-Algra (2004) as normal-optimal, normal-suboptimal, mildly abnormal, and definitely abnormal. It has been shown the early normal or abnormal findings of the GMs quality are highly predicative for later outcome (Hadders-Algra).

4D ultrasound also made possible the ability to study a full range of facial expressions such as smiling, crying, scowling, and eyelid movements. Observations of these movements may be of scientific and diagnostic value (Kurjak, Azumendi, Andonotopo, & Salihagic-Kadic, 2007; Kurjak et al., 2008). Evaluation of the structure and function of the facial movements is significant because the same inductive forces cause the growth and reshaping of the neuronal tube and the face. In addition, many genetic disorders of the CNS are characterized by dysfunction and dysmorphology of facial structures (Kurjak et al., 2003, 2004). Facial movements are included in the scoring system of the KANET as they reflect brain development (Kurjak, Miskovic, et al., 2007a).

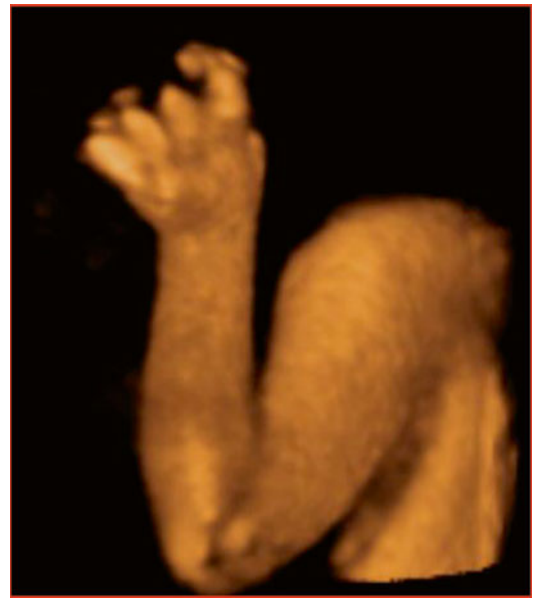

Fig. 10.1 3D ultrasound, display of the fetal arm with the neurological thumb in a clenched fist

Finally, KANET assessment includes: isolated arm anteflexion, overlapping cranial sutures and head circumference, isolated eye blinking, facial alteration, mouth opening, isolated hand and leg movements, hand-to-face movements, finger movements and thumb position, and a Gestalt perception of general movements (see Figs. 10.1 and 10.2; Kurjak et al., 2008). The standardization of the KANET test was proposed in Osaka, Japan, during the meeting of the International Academy of Perinatal Medicine held in October 2010. Subsequently, eight of the ten parameters should be used in the scoring because facial and mouth movements are combined into one category and also isolated hand movements and hand-to-face movements form a common category. In this way, a smaller number of parameters was achieved. The KANET should be performed using 4D ultrasound in the third trimester from the 28th to the 38th week of

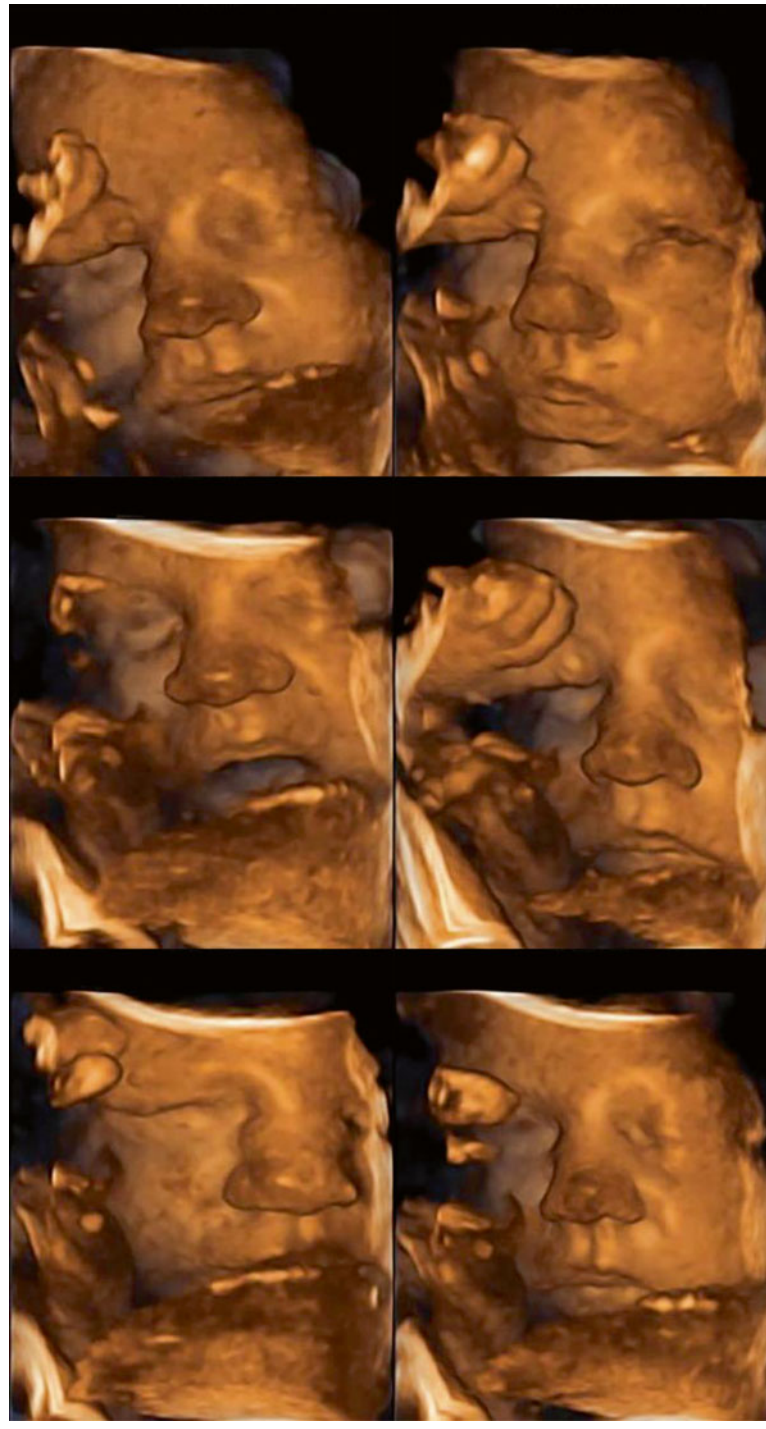

Fig. 10.2 Display of the fetus using 4D ultrasound in the 38th week of pregnancy. The KANET score was normal. Images show opening of the eyes, mouth opening, movement of the leg, and head rotation

gestation. Fetuses should be awake while examined and the assessment should last from 15 to 20 min. If the fetus is asleep, the examination should be postponed for 30 min or for the next day between 1400 and 1600 h. In cases of a definitely abnormal or borderline score, the test should be repeated every 2 weeks until delivery. The test score ranges from 0 to >14; for neurologically abnormal fetuses the range is 0–5; a borderline score is from 5 to 13; and a normal score is 14 or above (Stanojević et al., 2011).

Published Data of the KANET Assessment

During several years the KANET was applied in almost 2000 fetuses between 20 and 38 weeks of gestation. The results of the assessments can be considered reliable since they have proven to be statistically reproducible over studies.

The first application of KANET was in 2008, assessing a group of 100 low-risk pregnancies (Kurjak et al., 2008). Postnatal neurological assessment was performed after delivery. All newborns judged to be normal attained a score between 14 and 20, which was assumed to be a score of optimal neurological development. Subsequently, the same scoring system was applied in a group of 120 high-risk pregnancies. Three subgroups of newborns were detected based on postnatal neurological findings: normal, mildly or moderately abnormal and abnormal. Normal neonates had a prenatal score between 14 and 20, mildly or moderately abnormal 5–13, whereas those infants who were assigned as neurologically abnormal had a prenatal score of 0–5. Ten fetuses who were postnatally described as mildly or moderately neurologically abnormal, achieved a prenatal score of 5–13, another ten fetuses postnatally assigned as neurologically abnormal had a prenatal score of 0–5. Among this later group of ten, four fetuses had alobar holoprosencephaly, one had severe hypertensive hydrocephaly, one had thanatophoric dysplasia and four fetuses had multiple malformations. These preliminary results demonstrated the ability of the KANET to identify abnormal behavior in severely neurologically damaged fetuses (Kurjak et al., 2008).

Studies have been carried out in several collaborative centers (Zagreb, Istanbul, Bucharest, and Doha) to evaluate the new scoring test (Kurjak et al., 2010). The objective of this multicenter study was to better define normal and abnormal fetal neurological function in utero in order to better predict, antenatally, which fetuses are at risk for adverse neurological outcome. The study included 228 fetuses from high-risk pregnancies. Definite abnormal KANET scores were identified in 18 fetuses, 6 of whom died in utero and 5 were terminated. Postnatal neurological assessment of the seven remaining fetuses by Amiel-Tison's method revealed 3/7 newborns to be abnormal (arthrogryposis, vermis aplasia and neonate of the mother with the previous child with cerebral palsy), while four were considered normal (ventriculomegaly, preeclampsia, thrombophilia, oligohydramnios). The three fetuses with both prenatal abnormal KANET scores and postnatal abnormal neurological status had especially reduced facial movements; the faces were like masks during repeated scans. Fetuses with vermis aplasia and arthrogryposis had normal cranial sutures but the isolated head flexion was small in range for both cases. Isolated hand movements, hand-to-face and leg movements were poor in repertoire for all three cases. The finger movements were cramped and invariable in all three fetuses. The Gestalt perception of GMs also was abnormal in these fetuses. In this study, the behavior of a fetus with acrania also was followed longitudinally. It was observed that the fetus had hypertonic movements with high amplitude and high speed at 20 weeks of gestation. The movements emerged abruptly with burst-pause patterns; the variability of head movements was missing; and, there were no changes in facial expressions. As gestational age advanced and the motor control was shifting from a lower to upper control center, the movement patterns also changed. At the gestational age of 32 weeks the fetus had no facial expressions (mask-like face) and the hand movement repertoire was very poor. At 36 weeks, the absence of both facial expressions and limb movements was observed.

Abnormal behavior patterns, as a result of lack of the appropriate control of the upper cortical centers on the motor activity, were clearly documented (Kurjak et al., 2010).

Another study confirmed the difference in fetal behavioral patterns between the fetuses from low-risk and high-risk pregnancies (Miškovic et al., 2010). Statistically significant differences for eight out of ten parameters of the KANET were shown: isolated anteflexion of the head, eye blinking, facial expressions (grimacing, tongue expulsion), mouth movements (mouthing, jawing, swallowing), isolated hand movement, hand-to-face movement, fist and finger movements, and general movements. Moderate correlation of the KANET and the ATNAT also was confirmed (Miškovic et al., 2010).

A group in Khartoum applied the KANET to a large number of fetuses during a 1 year period in a prospective longitudinal cohort study (Talic et al., 2011). The aim of the study was to assess the behavior by application of the KANET scoring test in a large sample of fetuses from normal and high-risk pregnancies and to compare the scores between those two groups. The KANET was applied in 620 singleton pregnancies between 26 and 38 weeks of gestation. There were 520 pregnant women in the high-risk group and 100 pregnant women in the low-risk group. The fetuses with congenital anomalies and multiple pregnancies were excluded from the study, in contrast to previous studies. The high-risk group was divided into subgroups: threatened preterm delivery with or without preterm premature rupture of membranes (PPROM), previous child diagnosed with cerebral palsy, hypertension in pregnancy with or without preeclampsia, diabetes before pregnancy or gestational diabetes, intrauterine growth restriction, polyhydramnios, Rh isoimmunization, placental bleeding, and maternal fever (>39 °C). KANET scores of fetuses in the low- and high-risk pregnancies were compared and the difference was statistically significant. The differences of KANET scores of the fetuses from the low-risk group compared with the following subgroups of the high-risk group were statistically significant: previous child diagnosed with cerebral palsy, hypertension (RR >160/100 mmHg), threatened preterm delivery, maternal fever, intrauterine growth restriction (IUGR), Rh iso-immunization, placental bleeding. KANET scores also differed significantly when comparing threatened preterm delivery with PPROM vs. threatened preterm delivery without PPROM; hypertension >160/100 mmHg vs. hypertension <160/100 mmHg; diabetes before pregnancy vs. gestational diabetes; IUGR with decreased resistance Index (RI) of Middle Cerebral Artery (MCA) vs. IUGR without decreased RI of MCA; and Rh isoimmunization without hydrops fetalis vs. Rh isoimmunization with hydrops fetalis. Among the fetuses with abnormal KANET score, one of the most represented were the fetuses from the threatened preterm delivery group. Comparison of individual KANET parameters between the fetuses from the low and high-risk pregnancies showed statistically significant differences for overlapping cranial sutures and the head circumference, isolated eye blinking, facial expressions (grimacing and tongue expulsion), mouth movements (yawning and mouthing), isolated hand movements, isolated leg movements, hand-to-face movement, finger movements, and GM. For isolated head anteflexion, the difference was not statistically significant. Until now, this has been the study with the largest number of fetuses (620) in which the prenatal KANET test was applied. The study demonstrated the potential of the KANET to detect and discriminate normal from borderline and abnormal fetal behavior in normal and in high-risk pregnancies (Talic et al., 2011).

In 2011, a case of successful prediction of normal early neurological development of the neonate by the KANET was reported. Normal early neurological status was confirmed with two tests (Amiel-Tison's Neurological Assessment and assessment of General Movements using the Prechtl method—Prechtl, 1990) despite the unfavorable intrauterine conditions, fetal growth restriction, and hypoxemia (Predojevic, Stanojevic, Vasilj, & Kadic, 2011).

Athanasiadis et al. (2013) evaluated fetal behavior and neurodevelopment and compared them in low- and high-risk pregnancies using the KANET in a prospective, comparative, cohort

study which included 152 pregnant women. The population was classified into low-risk pregnancies ($n=78$) and high-risk pregnancies ($n=74$) which were subdivided into three groups: IUGR fetuses ($n=12$), diabetes mellitus ($n=24$), and preeclampsia ($n=38$). Statistically significant higher neurodevelopmental scores was revealed in the low-risk group. The diabetic subgroup scores were higher compared to the two other subgroups (IUGR and preeclampsia). It was concluded that the assessment of fetal neurodevelopment by 4D ultrasound appears to be a feasible method in the evaluation of high-risk pregnancies (Athanasiadis et al., 2013).

In a prospective cohort study at Women's Hospital in Doha, a population of 80 pregnant women was included (Abo-Yaqoub, Kurjak, Mohammed, Shadad, & Abdel-Maaboud, 2012). There were 40 pregnancies between 20 and 38 weeks of gestation with high-risk for fetal neurological abnormalities and 40 low-risk pregnancies. The goal was to determine the role of 4D ultrasonography in prenatal assessment of fetal neurobehavior and in the prediction of adverse neurological outcome. The KANET was employed for prenatal neurological assessment of the fetus and postnatal neurological assessment was performed using Amiel-Tison's Neurological Assessment at Term for all live-borns. All the cases of abnormal KANET scores were confirmed postnatally. The difference in the range of KANET scores was significant. A significant difference was shown for all KANET parameters except isolated leg movement and cranial sutures (Abo-Yaqoub et al., 2012).

Interesting results were published by Talic et al. (2012) whose aim was to assess differences in fetal behavior in normal fetuses versus fetuses with cerebral ventriculomegaly (VM (Talic et al., 2012). Over a period of 18 months, a longitudinal prospective cohort study was conducted and KANET was applied to evaluate fetal behavior. After assessment of 100 normal fetuses in the control group and 140 fetuses diagnosed with VM, the following results were obtained: there were 2 fetuses with abnormal KANET scores in the control group and 37 fetuses in the group with VM. The largest number of abnormal KANET scores was found in 22 fetuses with severe VM accompanied by other structural abnormalities. A borderline KANET score was found in 4 fetuses in the control group and 12 fetuses in the group with VM. Statistical analysis showed a significant difference between KANET scores of the two groups. There were no fetuses with abnormal KANET score in the group of isolated mild and moderate VM. On the other hand, the difference between KANET scores was obvious in the fetuses with severe isolated VM and in mild, moderate and severe VM with additional congenital CNS malformations. The authors (Talic et al., 2012) concluded that evaluation of the behavior in fetuses with cerebral VM using the KANET has the potential to detect fetuses with abnormal behavior and to add the dimension of CNS function to the morphological criteria of VM. Long-term postnatal neurodevelopmental follow-up is needed to confirm the data from prenatal investigation of fetal behavior (Talic et al., 2012).

In a recent study, the relation between fetal behavior and the circulatory changes in umbilical and cerebral arteries was assessed (Kurjak, Talic, Honemeyer, Stanojevic, & Zalud, 2013). There were 273 low-risk and 596 high-risk fetuses observed. High-risk pregnancies were divided into subgroups according to the following risk factor: pregnancy-induced hypertension, fetal growth restriction, gestational diabetes mellitus, threatened preterm birth, antepartum hemorrhage, maternal fever, sibling with cerebral palsy, Rh immunization, and polyhydramnios. Statistically significant differences in the distribution of normal, borderline and abnormal KANET scores were found between the low- and high-risk fetal groups. The largest number of fetuses with abnormal KANET scores was found in the subgroup of fetuses whose siblings were diagnosed with cerebral palsy (23.9 %), followed by the borderline KANET scores subgroup of fetuses from febrile mothers (12.7 %). Fetal behavior was significantly different between the low-risk group and the following high-risk subgroups: fetal growth restriction (FGR), gestational diabetes mellitus, threatened preterm birth, antepartum hemorrhage, maternal fever, sibling with cerebral palsy, and polyhydramnios (Kurjak,

Talic, Stanojevic, et al., 2013). This was the first study to analyze the deviations in fetal behaviors assessed by the KANET in relation to the circulatory changes in umbilical and cerebral arteries in different categories of high-risk pregnancies. Significant differences were found in fetal behavior when circulatory changes in the fetal brain were present. It was suggested that the alterations in fetal behavior occurred prior to the redistribution of the circulation towards the fetal brain in conditions of hypoxia.

A case report followed that assessed the neurological status of five fetuses with severe intrauterine growth restriction by combining the observation of hemodynamics and the motor activity of the fetus (Predojević, Talić, Stanojević, Kurjak, & Salihagić Kadić, 2014). In all cases in which the Doppler hemodynamic parameters indicated hypoxia, KANET scores was abnormal; in one case, where there was no significant redistribution of blood flow in favor of the fetal brain, the KANET score was borderline. In this study, KANET provided important information on brain function, while hemodynamic parameters informed us of the fetal cardiovascular response to hypoxia, which includes the redistribution of fetal blood flow to vital organs, especially the brain. Hence, a combination of motoric and hemodynamic parameters provided better insight into fetal well-being and neurological status of the growth restricted fetuses.

In 2013, the study of the KANET was expanded for the first time to twin pregnancies (Kurjak, Talic, Stanojevic, et al., 2013). The aim was to assess the onset and frequency of the first intertwin contacts by 4D ultrasound in the first trimester of pregnancy and to compare fetal behavior and KANET scores of twins and singletons in the second and the third trimesters. It was observed that, with increasing gestational age, a higher frequency of movements was noted. The number of abnormal, borderline, and normal KANET scores between singletons and twins was not statistically significant. Scores for isolated eye blinking, mouthing, grimacing, hand-to-head movement, finger movements, Gestalt perception, and GM differed significantly in twins and singletons.

Conclusion

Appreciating the fact that fetal behavior patterns directly reflect the development of the central nervous system, it is important to know the normal range and types of movements so that we can distinguish normal from abnormal behavior in utero. Toward this aim, researchers have been using the KANET test which has led to great progress in the perinatal estimation of fetal neuromotor development and neurological status. However, further studies are necessary as well as long-term monitoring of children who were assessed by the KANET in order to determine its clinical value in predicting prenatal neurological risk in children.

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The Potential Value of Habituation in the Fetus

11

Leo R. Leader

Abstract

This chapter reviews studies of habituation in the fetus. It covers the history of habituation and its relationship to altered function of the central nervous system (CNS) as well as the different ways of measuring it. It outlines how factors such as gestational age, decreased O_2 tension, cigarette smoking, drugs and alcohol effect habituation. It examines the differences in habituation patterns seen in both high-risk fetuses and those with Down's syndrome and their possible predictive value. The question of the safety of its use is also discussed. The chapter includes new studies of the possible clinical value of using habituation to repeated vibroacoustic/sound stimuli (VAS). Data are presented on the ability of fetal habituation to predict infant development at 36 months of age as well as at 7–8 years. Also presented is a new form of fetal heart rate analysis called functional regression analysis which offers the exciting possibility of predicting, whilst still in utero, the infant's development at 18 months of age and at 3 years.

Keywords

Habituation • Fetal • Vibroacoustic stimulation • Infant development • Functional regression analysis

Introduction

This chapter reviews studies of habituation in the fetus. It raises the question of fetal compensatory mechanisms that occur during stress and the value of testing for fetal wellbeing under resting conditions (without using stimulation of any kind). This is the usual way fetal well-being is currently assessed. There are many new studies of the possible clinical value of using habituation to repeated vibroacoustic/sound stimuli (VAS). It covers the history of habituation and its relationship to altered function of the central nervous system (CNS) as well as the different ways of measuring it. It outlines how factors such as gestational age, decreased O_2 tension, cigarette

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smoking, drugs and alcohol effect habituation. It examines the differences in habituation patterns seen in both high-risk fetuses and those with Down's syndrome and their possible predictive value. The question of the safety of its use is also discussed. Data are presented on the ability of fetal habituation to predict infant development at 36 months of age as well as at 7–8 years. Also presented is a new form of fetal heart rate analysis called functional regression analysis which may offer the exciting possibility, whilst still in utero, of predicting the infants development at 18 months of age and at 3 years.

One of the major challenges still facing clinicians dealing with "high-risk" pregnancies (e.g., those complicated by conditions such as intrauterine growth retardation (IUGR) or severe hypertension) is determining the optimal time for delivery to try and avoid any risk not only of serious perinatal complications but also to reduce the possibility of minimal brain damage. It has been shown that the incidence of minor neurological dysfunction (MND) was 16 % in normal infants and up to 40 % in preterm infants born small for gestational age (SGA) (Hadders-Algra & Touwen, 1990).

Management of High-Risk Pregnancies

In the management of these "high-risk" pregnancies, provided the clinical situation does not pose a serious threat to the mother, the pregnancy is usually allowed to continue to achieve the maximal maturity. These fetuses are monitored by asking the mother to record fetal movements, measuring the variability of fetal heart rate using a cardiotocograph (CTG) or a combination of observations in a biophysical profile using real-time ultrasound. Many units are using Doppler flow velocity waveforms in the umbilical artery and middle cerebral artery to assess fetal well-being.

Delivery is still usually indicated if one or more of the above tests are abnormal as there is a strong association between abnormal results and perinatal complications such as asphyxia, acidosis and admission to special care units. Most of these observations are made with the fetus at rest without using any form of fetal stimulation.

Reserve Mechanisms

Most physiological systems have compensatory mechanisms which begin to function only when the organism is stressed or finds itself under suboptimal conditions. In human fetuses, it has been shown using Doppler flow velocity studies, that in the presence of hypoxia, the resistance index in the carotid vessels falls to preserve the blood flow to the fetal brain. This is associated with an increase in the resistance in the umbilical vessels (Arias & Retto, 1988). Thus, a test performed under resting conditions may be of only limited predictive value as these reserve mechanisms may be functioning and give an apparently normal test result.

A similar situation is seen in adults with heart disease. In adults, an electrocardiograph (EKG) done with a patient lying in bed may be normal but when the patient is placed on a treadmill and exercised (stressed) the EKG may show marked abnormalities.

Effect of Stress

To try and test the limits of any system, it needs to be challenged or stimulated. Selye (1976) described the General Adaptation Syndrome (GAS) which outlines a tripartite physiological-behavioral response to stress: (1) an alarm reaction; (2) a stage of resistance (adaptation) during which time the organism's defence mechanisms are mobilized and symptoms are alleviated; and, (3) should the stress situation continue beyond the organism's finite level of "adaptation energy," exhaustion occurs and changes will be detectable under resting conditions. This concept is reviewed by Szabo, Tache, and Somogyi (2012).

Stimulation

Stimulation can be used in two ways: (1) by measuring the response to a single stimulus or (2) by observing the decrease in response to repeated stimulation (i.e., habituation). A test which elicits a behavioral response will provide more information as it involves both sensory and motor

responses which require a higher degree of neuronal involvement. This is supported by the observation that although anencephalic fetuses may have normal movement activity and resting CTG recordings, they do not show a response to VAS (Leader, Baillie, Martin, & Vermeulen, 1982a; Ohel, Birkenfeld, Rabinowitz, & Sadovsky, 1986) nor do they habituate (Brackbill, 1971). This is also the rationale for the increasing use being made of CTG changes following trans abdominal VAS to assess fetal well-being.

Ideal Prenatal Test

Currently used measures such as CTG, biophysical, and Doppler blood flow measurements which have been outlined above are indirect and assess general well-being which has only an indirect relationship to cortical function, the ultimate arbiter of excellence in man. Translated into more practical terms, the ideal test of well-being in the fetus should provide a precise measure of CNS integrity. Habituation may be such a test.

Habituation

Habituation is the decrease leading to cessation of a behavioral response that occurs when an initially novel stimulus is presented repeatedly (Thompson & Glansman, 1966). Although habituation is remarkably simple it is one of the most widespread forms of learning (Buckwald & Humphrey, 1973; Kandel, 1979; Stevenson & Siddle, 1983). Overt activity reflects only a minor part of the information processing by the CNS. In an environment of constant sensory stimulation, this ability to ignore meaningless stimuli is essential for the efficient functioning and survival of the organism.

There is good evidence that a normal habituation pattern reflects an intact and fully functioning CNS (Jeffrey & Cohen, 1971; Joy, McClure, Hepper, & Cooke, 2012; Leader & Bennett, 1995; Lewis, 1971; Madison et al., 1986; Sokolov, 1977; Wyers, Peek, & Herz, 1973).

Controlling Mechanisms

It is not known which part of the CNS controls habituation but there are data to suggest that the functioning intact CNS is essential for normal habituation (Joy et al., 2012; Leader & Bennett, 1995; Sokolov, 1977). A recent study in human adults supports the hypothesis that cortical inhibition plays a role in the mechanism of habituation (Palermo et al., 2011).

Prenatal Observation of Habituation

Peiper (1925) first noted cessation of the fright response to repeated sound using an automotive horn sounded near the maternal abdomen. Fleischer (1955) noted habituation of fetal movements to repeated sound stimulation. Leader et al. (Leader et al., 1982a; Leader, Baillie, Martin, & Vermeulen, 1982b) examined habituation of the fetal movement response to repeated VAS. Habituation of the blink-startle response was described by Birnholz and Benacerraf (1983). Madison, Adubato, et al. (1986) also demonstrated habituation of the fetal movement response to repeated VAS. Similar observations were made by others (Kuhlman, Burns, Depp, & Sabbagha, 1988; Shalev, Benett, Megory, Wallace, & Zuckerman, 1989). More recently a number of studies have shown prenatal habituation of the movement response to repeated stimulation using sign waves with a frequency of either of 250 or 500 Hz. They have refined the response by establishing the intensity at which each individual fetus responds and using that intensity to demonstrate habituation (Dirix, Hornstra, & Nijhuis, 2009; Dirix, Nijhuis, Jongsma, & Hornstra, 2009; Hepper, Dornan, & Lynch, 2012b; Joy et al., 2012; Shahidullah & Hepper, 1993). Smotherman and Robinson (1992) originally demonstrated habituation of the heart rate and movement response to repeated chemosensory stimuli in fetal rats using infusions of a lemon solution onto the fetal tongue. There have been many studies that have shown habituation of the fetal heart response to VAS (Kisilevsky & Hains, 2010; Ratcliffe, Heller, & Leader, 2002a;

Ratcliffe, Leader, & Heller, 2002b). Fetal habituation using magnetoencephalocography (MEG) has been demonstrated to both the visual stimuli (Matuz et al., 2012) and to auditory stimuli (Muenssinger et al., 2013).

Habituation and Altered CNS Function

According to Lewis (1971), a normal habituation pattern reflects an intact CNS. Deviations from normal CNS function have altered habituation in that: brain damage produces a reduction of behavioral response habituation (Holloway & Parsons, 1971). Schizophrenics have different habituation patterns compared to normals (Gruzelier & Venables, 1972) as do patients suffering dementia due to cortical atrophy and Parkinson's disease.

Hyperactive children have impaired habituation to visual, tactile, and auditory stimuli (Hutt & Hutt, 1964; Tizard, 1968). Autistic children do not show normal behavioral and electroencephalographic habituation (Hutt, Hutt, Lee, & Ounsted, 1965). Different habituation patterns have been found between children with Down's Syndrome and normal controls (Dustman & Callner, 1979; Hepper & Shahidullah, 1992). Anencephalic infants also fail to habituate (Brackbill, 1971). Van Heteren, Focco Boekkooi, Jongsma, and Nijhuis (2001) showed that a fetus with trisomy 18 failed to respond to VAS and the same authors (van Heteren, Boekkooi, Jongsma, & Nijhuis, 2000b) showed that responses to VAS in a fetus with an encephalocele was different to responses of normal fetuses.

Maternal ingestion of alcohol (Hepper, Dornan, & Lynch, 2012a) and cigarette smoking alter the fetal ability to habituate. In humans, prenatal exposure of the fetus to antipsychotic drugs alters its ability to habituate at 6 months of age (Figueras et al., 2011).

In animal studies, drugs known to effect the CNS such as amphetamines (Davis, Svensson, & Aghajanian, 1975), lysergic acid diethylamide (Key, 1961), barbiturates and chlordiazepoxide (Lader & Wing, 1965) alter habituation.

Fetal Habituation

In the studies outlined below, a broad spectrum VAS generated by a Ronson's electric toothbrush was used. The fetal movement response was assessed by a Hewlett-Packard CTG which was modified to reduce the filtering on the tocodynamometer which increased its sensitivity to detecting fetal movements, a real time ultrasound watched by an independent observer and maternal observation.

The toothbrush was placed on the maternal abdomen over the fetal head and a 5-s stimulus applied approximately every 20 s. Movement that occurred either during the stimulus or within 2.5 s of its cessation was regarded as a response. Movement after that time was regarded as spontaneous. If there was spontaneous movement when the stimulus was due, it was withheld until the movement ceased.

Lack of response to five consecutive stimuli indicated habituation and the number of stimuli required to produce habituation was recorded. These criteria were similar to those described in the newborn infant by Brackbill, Kane, Manniello, and Abramson (1974) and Madison, Adubato, et al. (1986) in human fetuses. It has been suggested that the probability of five consecutive observation periods without any fetal movement would occur by chance in less than 5 % of the time.

Participant Selection

Normal Range

To establish a normal range, 40 subjects (Leader et al., 1982a) who had a normal uncomplicated antenatal, intrapartum, and neonatal course and were delivered of infants thought to be in optimal condition were used as controls (Leader et al., 1982a; Michaelis, Rooschüz, & Dopper, 1980). These subjects were determined retrospectively.

The optimality concept was suggested by Prechtl (1980) and places the emphasis on finding the best possible conditions rather than

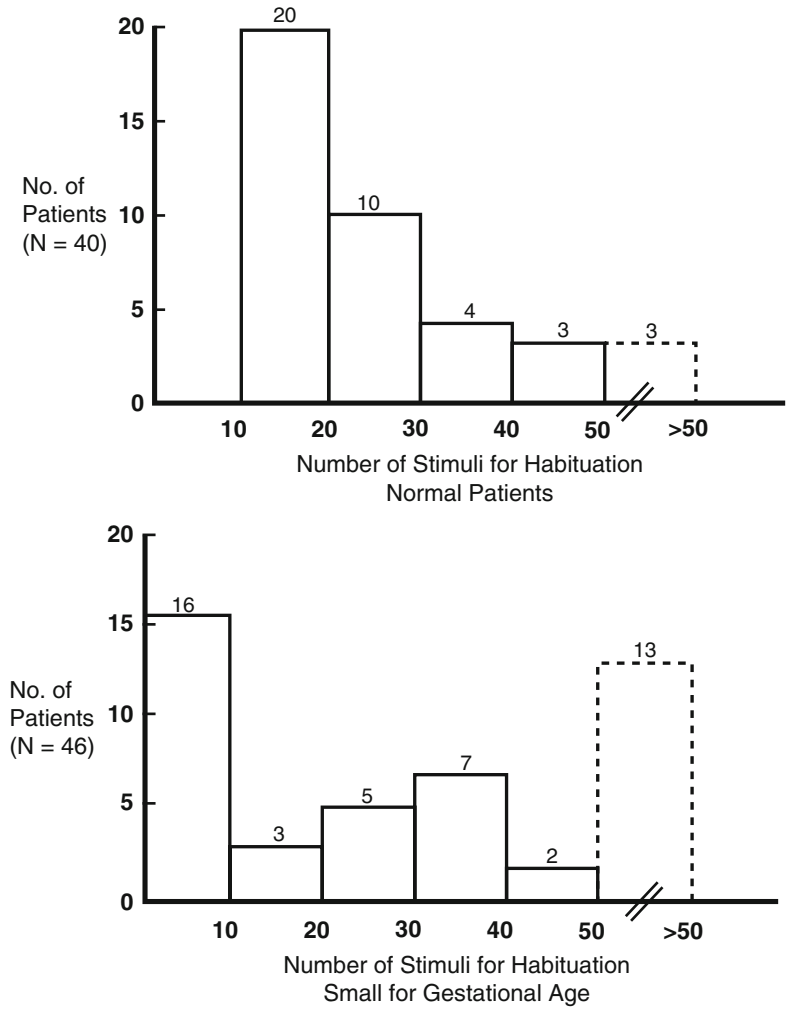

Fig. 11.1 Habituation patterns in optimal fetuses ($n=40$) and those born small for gestational age ($n=46$). *Dashed line* Fetuses that fail to habituate by 50 stimuli

normality, abnormality, or pathology. An optimal condition is more restrictive and is not synonymous with normality. It is more narrowly defined (e.g., a teenage patient having her first baby may well be considered normal but would not be considered optimal because of the well-known increased mortality rates compared to a patient in her 20s having her second or third baby). Figure 11.1 shows that 37 of the 40 patients studied, habituated after 10–50 stimuli, and 34 (85 %) habituated in 40 or less. This is similar to a previous study in which normal neonates took between 20 and 37 stimuli to habituate to a broad-spectrum sound stimulus (Eisenberg, Coursin, & Rupp, 1966).

High-Risk Pregnancies

The optimal group were then compared to 46 patients whose infants had birth weights below the 10th percentile for the local population and were classified as SGA (Leader et al., 1982b). See Fig. 11.1, bottom panel. In that group, 8 fetuses failed to show any response to stimulation (non responders); 8 habituated between 1 and 9 stimuli; 17 were in the normal range; and, 13 failed to habituate after 50 stimuli. Similar habituation patterns were seen in a further 38 who had decreased growth velocity as assessed by serial ultrasound observations of their biparietal diameters and in 28 patients who had meconium staining of the

amniotic fluid as well as in a further 28 fetuses who had normal growth on their serial ultrasound biparietal diameters. This was a mixed group consisting of some normal pregnancies and some complicated by hypertension, ante partum haemorrhage and decreased fetal movements. This suggests that in some at risk pregnancies, the presence of normal fetal growth may not mean that all is well. There were highly significant differences between the optimal control group and the high-risk groups ($p < 0.0005$).

Habituation Patterns

Leader et al. (1982a, 1982b) were able to identify four different patterns of response: (1) normal habituators who take between 10 and 50 stimuli; (2) non-responders—a group that failed to respond to stimulation; (3) fast habituators who habituated between 1 and 9 stimuli; and, (4) slow habituators who were still responding after 50 stimuli. The study included five patients whose infants had major CNS abnormalities, four anencephalics and one microcephalic. All five infants failed to respond to stimulation.

Dishabituation

Dishabituation classically refers to the recovery of a habituated response to the original stimulus following the presentation of a novel stimulus. This process is very important as it differentiates between neural or receptor fatigue and habituation. In the study outlined above, dishabituation was demonstrated using a second mechanical vibrator and was noted in 83 % of normal subjects. Dishabituation also has been demonstrated by many authors (Hepper et al., 2012a; Hepper & Shahidullah, 1992; Madison, Adubato, et al., 1986; Muenssinger et al., 2013). Smotherman and Robinson (1992), in studies in fetal rats, also have demonstrated dishabituation.

These studies have all clearly demonstrated that the process is assessing habituation and not neural fatigue. Provided authors use a interstimulus interval of 20 s or longer, it is not necessary for every study to demonstrate dishabituation. Using a long interstimulus interval makes it very unlikely that neural fatigue will occur and lead to failure to respond (Thompson, 1992). It is more likely to occur when the stimulus is applied at millisecond intervals.

Factors Effecting Human Fetal Habituation

Gestational Age

To determine the gestational age of onset of habituation to a VAS, the fetuses of 27 nonsmoking pregnant women were studied at 2 weekly intervals from approximately 22 weeks of gestation to determine at what age the human first responded and habituated (Leader et al., 1982a). At 23–24 weeks, only 7 % of fetuses responded. By 27–28 weeks, 89 % of them responded. The onset of the response occurred earlier in females than males. 75 % of the 12 females responding by 25–26 weeks compared to only 33 % of the 15 males. All females responded by the 28th week whilst 80 % of the males responded by then. By 30 weeks, all fetuses responded. This is in keeping with known neuro-physiological data that female infants mature earlier than males (Singer, Westphal, & Niswander, 1968)

The blink startle response to a VAS was first noted at 24–25 weeks and was consistently present after 28 weeks (Birnholz & Benacerraf, 1983). Similar observations about the onset of fetal responsiveness and sex differences have now been made by others (Buss et al., 2009; Hepper, 1992; Hepper et al., 2012b; Kuhlman & Depp, 1988)

Effect of Repetition

Fifteen fetuses in normal pregnancies and more than 36 weeks of gestation were tested on 2 successive days. Fourteen of them required fewer stimuli for habituation. The sign test in this group was significantly negative ($p = 0.035$). The only fetus who showed no decrease was found to have meconium staining of the amniotic fluid when his

mother went into labour. Fetuses tested after intervals of 3–4 days, showed no consistent habituation pattern and a sign test was not significant (Leader & Bennett, 1995). This was the first study that found that there is some evidence of fetal memory which lasts for 24 h. When tested after 72 h there was no evidence of such an effect.

van Heteren, Boekkooi, Jongsma, and Nijhuis (2000a) also using an habituation paradigm confirmed the presence of fetal memory lasting up to 24 h. They, however, in contrast to most other studies of habituation used a failure to respond to four consecutive stimuli as their criterion for habitation rather than five stimuli. Using VAS and an habituation paradigm other researchers (Dirix, Hornstra, & Nijhuis, 2009; Dirix, Nijhuis, Jongsma, & Hornstra, 2009) have demonstrated that normal human fetuses from 30 weeks of gestation onwards display short term memory (10 min) and that, by 38 weeks gestation, the fetus may have developed a 4 week memory span.

Cigarette Smoking

To test the effects of maternal smoking on fetal habituation, we recruited a number of pregnant women who smoked and tested their fetuses for habituation (Leader, 1987).

Eight pregnant women were tested more than 1½h after their last cigarette. All eight fetuses had a normal habituation pattern (10–50 stimuli). When the same women were tested 3–7 days later, less than an hour and a half after smoking, seven fetuses had an abnormal habituation pattern (<9 or >50 stimuli). The remaining woman, who smoked 40 cigarettes a day required 6 h before fetal habituation returned to normal (Leader, 1987).

A further study (Leader, 1987) also examined the effects of smoking. The fetuses of 13 nonsmoking women who were more than 36 weeks pregnant were tested for habituation. Women remained recumbent on their left sides on the examination couch. After a 30-min break, fetuses were retested for habituation and the test repeated for the third time after a further 20-min break. The fetuses of nine pregnant women who were smokers, all of whom had refrained from smoking for 6 h, were tested using a similar protocol. It differed, however, in that mothers were asked to smoke two cigarettes at the end of their second trial.

Figure 11.2 shows that in nonsmokers, there was a progressive decrease in the number of stimuli required for their fetuses to habituate after the third trial (first trial 27.08±2.63, second 21.61±5.6, third 15.38±3.91). In smokers, there was also a decrease in the number of stimuli to habituate (first trial 30.00±5.14, second 16.00±5.15) but after smoking two cigarettes fetuses took significantly ($p=0.006$) longer to habituate (third trial 31.22±5.22). Fetuses of smoking women took significantly longer to habituate than those of nonsmokers after three trials.

In addition, Hepper (1992) has shown that fetuses whose mothers smoke require a greater intensity of stimulus to evoke a response compared to those mothers who do not smoke.

Effects of Drugs

Following the observation that adult patients on sedatives tended to have an abnormal habituation pattern, nine normal pregnant subjects, who were more than 36 weeks of gestation were recruited and their fetuses tested for habituation (Leader & Bennett, 1995). The women consented to take phenobarbitone 30 mg 8 hourly for 3 days. The fetal habituation test was then repeated after 4–5 days. Where possible, a third test was done on the 7th or 8th day.

A group of 14 women with similar characteristics acted as controls and their fetuses were tested at approximately the same intervals. They did not, however, receive any barbiturates.

In the nine fetuses, whose mothers took barbiturates, all nine had a normal habituation pattern before commencing any drugs. When the test was repeated on day 4, only three had a normal pattern. Seven of the 13 when tested on day 4 or 5 failed to habituate by 50 stimuli. When they were tested after stopping the sedatives, their habituation pattern returned to normal once again.

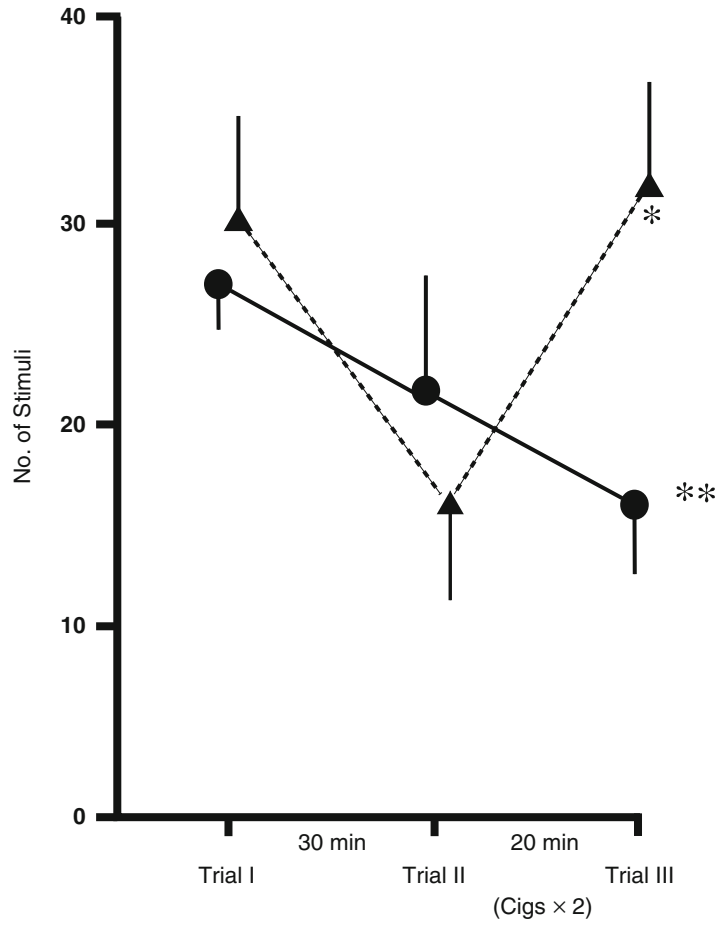

Fig. 11.2 Habituation rates in fetuses of nine mothers who smoked and 13 who did not. Smokers had two cigarettes in the 20 min between trial 2 and 3. *$p = 0.006$ Smokers trial 2 vs. trial 3. **$p = 0.02$ Trial 3 smokers vs. nonsmokers. *Filled triangle* = Smokers, *filled circle* = Nonsmokers

The fetuses in the control group showed no change in their habituation pattern. The controls differed significantly from the drug group when comparing day 4 alone or together with day 5 ($p < 0.05$).

Alterations in the Inspired Maternal Oxygen

Twenty three nonsmoking, normal pregnant women who were more than 36 weeks gestation were recruited (Leader & Baillie, 1988). The fetuses of ten women were initially tested for habituation while breathing room air and the test was repeated the following day at approximately the same time of day whilst the mothers were breathing 12 % oxygen. A second group of eight fetuses were tested while their mothers breathed 12 % oxygen on the first day and the fetal habituation test was repeated the next day whilst the women breathed room air. There was a third group of five women whose fetuses were tested while the women breathed room air on the first day and were retested the following day while breathing air through the same apparatus used to deliver the 12 % oxygen. This was to ensure that using the apparatus did not influence the fetal habituation test.

All ten subjects while breathing air on the first day had a normal habituation pattern compared to only one fetus who has a normal habituation test when their mothers were tested breathing 12 % oxygen. In the group who had 12 % oxygen the first day, only one fetus had a normal pattern and seven fetuses had abnormal patterns. When

the fetuses were tested the following day whilst their mothers breathed room air, seven out of the eight had normal habituation patterns.

In the controls, there were no significant differences seen in fetal habituation patterns when mothers were tested breathing air through the same apparatus. It is thus clear that altering the amount of inspired oxygen effects the habituation pattern on a temporary basis. Breathing 12 % oxygen is the equivalent of living at 13,000 ft above sea level and reduces the maternal PAO_2 from 99 to 44 mmHg. The arterial saturation, however, only falls to 86 %. Due to the sigmoid shape of the oxygen dissociation curve, decreasing the inspired oxygen concentration any lower than 12 % leads to a large fall in the oxygen saturation and hypoxia.

Down's Syndrome and Response Latencies

Hepper and Shahidullah (1992) reported two cases of Down's syndrome, one of whom failed to habituate and the other took longer to habituate than controls. They also determined the time from the onset of the stimulus to the initiation of movement. Normal fetuses showed a progressive decrease in latency over trials. One of the Down's fetuses who failed to show any change in its response latency died shortly after birth, although there was no detectable difference between the two antenatally.

Effect of State on Fetal Habituation

Until the study by Leader et al. (1982a), it was widely believed that the fetus had little capacity to respond to external stimulation and if it did, the responsiveness was dependent on fetal behavioral state (Prechtl, 1985; Schmidt, Boos, Gnirs, Auer, & Schulze, 1985). Those studies failed to take into account, however, the type of stimulus, its intensity, how it was delivered, nor does it take into account the effects of repetition (habituation). It was also suggested that the decreased responsiveness seen in infants was not habituation but a change in state (Hutt, von Bernuth, Lenard, Hutt, & Prechtl, 1968). Many studies have clearly demonstrated that fetal responsiveness increases with increasing stimulus intensity, frequency (Gagnon, 1989; Kisilevsky, Muir, & Low, 1989; Lecanuet, Granier-Deferre, & Busnel, 1989; Yao et al., 1990) and duration (Pietrantoni et al., 1991). These studies demonstrate that, if the stimulus is strong enough, it will override the effect of state.

Does Vibroacoustic Stimulation Effect Fetal State?

Because of the concern expressed by others on the effects of VAS on fetal state, two sets of experiments were done. Fetal state was determined according to the accepted criteria (Nijhuis, Prechtl, Martin, & Bots, 1982)

State 1f is usually associated with low heart rate variability and no eye or body movements. State 2f is associated with higher heart rate variability, accelerations, and the presence of eye and body movements. State 3f is usually associated with low heart rate variability but also the presence of fetal eye movements. State 4f is associated with prolonged accelerations, eye and excessive body movements.

In the first experiment only a CTG was used to determine fetal state according to the heart rate variability. This was similar to the method reported by others (Lecanuet, Granier-Deferre, Cohen, Le Houezec, & Busnel, 1986). The fetus was stimulated with three 5-s VAS and the changes in heart rate variability observed for 30 min after each stimulus.

Table 11.1 shows the heart rate variability before and after each of the three stimuli in 90 tests. Percentages are shown in parentheses.

As can be seen from the table, fetal stimulation was associated with a change in fetal state but the changes were short lived. The incidence of LV and HV did not differ significantly from each other before each of the three stimuli. There was also a decrease in the frequency of LV after stimulation. Although 15 % of fetuses had very reactive (similar to state 4) heart rate patterns after the second

Table 11.1 Average fetal heart rate variability before and after each of three vibroacoustic stimuli

	First stimulus		Second stimulus		Third stimulus	
	Before	After	Before	After	Before	After
HV	70(78)	72(80)	77(85)	63(70)	60(82)	63(88)
LV	20(22)	7(8)	12(13)	7(8)	12(13)	4(5)
LV to HV	0	3(3)	0	2(2)	0	2(3)
HV to LV	0	2(2)	0	4(4)	0	2(3)
VR	0	4(4)	1(1)	14(15)	2(3)	2(3)

HV high variability, *LV* low variability, *VR* very reactive with prolonged accelerations

stimulus, most of these fetuses reverted to either LV or HV over the 10 min before the third stimulus. Only 3 % had very reactive tracings after the third stimulus. This may represent another form of habituation to the stimulus.

In the second set of experiments, the effects of two different vibroacoustic stimuli and a sham stimulus were assessed on fetal state. The VAS used were the Ronson's electric toothbrush (Leader et al., 1982a) and the Corometrics Acoustic Stimulator. Fetal state was assessed using two real-time ultrasound scanners and a CTG as described by Nijhuis et al. (1982). There were approximately 60 h of observation in nine subjects. After a 30-min control period, three 5-s VAS were applied and the changes in fetal state observed for 30 min after each stimulus.

This study also found that, although there were some changes in fetal state, these were short lived and fetuses who were regarded to have changed to state 4 reverted back to either a state 1 or 2 before the next stimulus was due (Leader, Fawcus, & Clark, 1992). The tachycardias and atypical patterns described as frequent by Visser, Mulder, Wit, Mulder, and Prechtl (1989) were not seen.

This difference in findings by Visser et al. is likely to be due to the intensity and frequency of their stimulus. This is supported by the studies by Kisilevsky et al. (Kisilevsky et al., 1989; Kisilevsky, Fearon, & Muir, 1998) who found that both the intensity and the spectral characteristics of the stimulus influenced the fetal response. In addition van Heteren, Boekkooi, Jongsma, and Nijhuis (2001) also studied the effects of fetal behavioral states on fetal habituation and found the rate of habituation was not influenced by the fetal behavioral state. The same group have studied habituation in postterm pregnancies (van Heteren, Boekkooi, Schiphorst, Jongsma, & Nijhuis, 2001b). Both studies have once again used a failure to respond to four successive stimuli as their criterion for habituation. Most studies use a failure to respond to five stimuli.

Habituation in Fetal Sheep

Because of the suggestion that fetal responsiveness to auditory stimulation was state dependent, an animal model was established using fetal sheep (Leader, Stevens, & Lumbers, 1988). The fetus was stimulated using a mechanical vibrator attached to the maternal abdomen. This study also showed quite clearly that fetuses responded to stimulation and habituated during both high voltage (HV) and low voltage (LV) electrocortical activity. Although they took 13 stimuli in HV compared to 23 in LV, these differences were not statistically significant. Similar observations have been made in human fetuses (Shalev, Weiner, & Serr, 1990). As we found that the electromagnetic field that the mechanical vibrator produced was interfering with our fetal electroencephalographic (EEG) and electromyographic (EMG) recordings, we changed our experimental design (Leader, Smith, Lumbers, & Stevens, 1989)

A 5 ml suffusion of cold saline over a 3-s period through the catheter that had been sutured to the fetal neck at the time of surgery (see above) was used as a stimulus. The distal end of this catheter was fenestrated to allow the saline to run across the fetal skin.

Fetal response. Fetal movements that occurred during or within 2.5 s of the stimulus, were considered a response. If the fetus was moving spontaneously at the time the stimulus was to be given, it was withheld until the movement ceased. The stimulus was repeated at approximately 20-s intervals until habituation occurred. This was defined as lack of a movement response by the fetus to five successive stimuli (5,16). The number of stimuli required to produce habituation was recorded.

Hypoxia. A further study was done to examine the effects of maternal hypoxia on fetal habituation (Leader et al., 1989). Hypoxia was induced by placing a bag over the ewes head and altering the amount of inspired O_2 to 9 % + 3 % CO_2. This reduced the fetal PO_2 from a mean of 18 mmHg before stimulation to approximately 10 mmHg during the stimulation. Hypoxic fetuses habituated far more rapidly than controls (2.4 ± 1.3 vs. 16.7 ± 2.7 stimuli respectively, $p < 0.01$). Habituation was tested whilst infusing a solution of noradrenaline directly into the fetus to produce levels of noradrenaline consistent with those seen during hypoxia. During the noradrenaline infusion, fetuses habituated significantly more rapidly than controls (4.6 ± 0.8 and 14.5 ± 2.3 stimuli respectively, $p < 0.001$).

The effect of alcohol. Alcohol was infused into pregnant ewes to produce blood levels in the fetus of approximately 80-90 mg/100ml before stimulation. This resulted in more rapid habituation (7.25 ± 1.28 stimuli) compared to a control experiment 2–5 days before the alcohol (21.5 ± 3.57) and a further control experiment 2–5 days after the alcohol (17.5 ± 5.83).

Fetal alcohol infusion was associated with a significant decrease in spontaneous fetal movements. The most likely explanation for this response is that it is due to catecholamine release that occurs after small amounts of alcohol (Leader, Smith, & Lumbers, 1990). Fetuses habituated faster during an IV infusion of noradrenaline ($p = 0.0009$).

Hepper et al. (2012a) also have demonstrated altered habituation in the fetuses of mothers who drink alcohol during their pregnancies. The more they drink at one session, the greater the effect.

Fetal Heart Rate Habituation

Habituation of the fetal heart rate response to sound stimulation was described by Goodlin and Lowe (1974) and to VAS by Leader, Baillie, Martin, Molteno, and Wynchank (1984) but no real clinical application was made of this observation. There are now many studies exploring the relationship between the fetal heart rate and cognition (Kisilevsky & Hains, 2010). Because determining fetal movement habituation requires real-time ultrasound and two observers, we have recently been studying fetal heart rate habituation as this may have broader clinical application as most hospitals have free access to a cardiotocograph which is the only equipment required to measure heart rate habituation

Safety of Vibroacoustic Stimulation

The safety of using VAS has been questioned by Visser et al. (1989). They have observed that stimulation with an electronic artificial larynx (EAL) induces excessive fetal movements, a prolonged tachycardia, and non-physiological state changes (i.e., many fetuses spent more time in state 4F or in an episode non classifiable because of an atypical heart rate tracing).

Our own experience has been quite different perhaps because of the experimental design in that the fetus is only stimulated once the heart rate has returned to its baseline.

Sound levels. When measured in air, the EAL had a sound pressure level of 105 dB at a distance of 5 cm from the vibrating membrane. When the EAL was tested in pregnant ewes by means of a hydrophone implanted within the uterus the sound level was even higher (135 dB) (Gerhardt, 1989). The overall sound pressure level produced by the stimulus used in our laboratory was 74 dB and has been previously described (Leader et al., 1982a). Its frequencies also differ from the EAL. More recently, Muenssinger et al. (2013) measured the intensity of the sound stimulation next to an infant's ear after the sound travelled through an air-filled tube and found it to be 65 dB. The sound

pressure level in air was measured at 95 dB. This suggests that sound attenuation occurs during intrauterine sound transmission.

Visser et al. (1989) also express concern that the changes in heart rate may be due to sudden release of catecholamines and may be harmful in severely growth retarded fetuses and lead to intracranial bleeding. Using our less intense stimulus, we have found that these fetuses, if severely affected, do not respond to stimulation (Leader et al., 1984). In addition, Fisk et al. (1991) have demonstrated that, in the human fetus, VAS is not associated with a rise in adrenalin or noradrenaline.

Ohel, Horowitz, Linder, and Sohmer (1989) have shown that neonatal auditory function is normal following intrapartum VAS and Nyman, Barr, and Westgren (1992) also have shown that VAS does not have an adverse effect on infant development.

In addition, a recent review of ultrasound use in pregnancy has concluded that ultrasound scanning has been used as a diagnostic and screening tool in obstetric practice for over 50 years. There is no evidence of immediate or long-term harm to the developing fetus from exposure to B mode ultrasound (Aiken & Lees, 2012).

Predictive Value of Habituation

Lewis (1971) found that the rate of visual habituation at 1 year of age was significantly related to learning tasks and IQ at 44 months of age. Subsequent studies also have shown that performance on habituation tasks has been predictive of later cognitive function (Bornstein, 1989; Rose & Wallace, 1985).

Further evidence to support the value of habituation as having predictive value was provided by Leader et al. (1984). They found that infants who had normal intrauterine *fetal movement* habituation patterns scored significantly higher on the Griffiths Mental Developmental Scale at 1 year of age compared with those who had abnormal intrauterine habituation patterns. In a further study of *fetal movement* habituation, which confirmed our original observations, fetal movement habituation was found to be clearly associated with postnatal behavior using the Brazelton Scale after birth (Madison, Madison, & Adubato, 1986). At 4 months of age, using the Bayley Scales of Infant Development, faster habituators were found to have significantly higher mental development scores.

Fast habituators probably represent the group in a stage of resistance described by Selye (1976) in his General Adaptation Syndrome (see above). Supporting this theory is the observation that fetuses who had a mixed heart rate pattern of accelerations and decelerations (which may reflect mild stress) in labour had a superior developmental outcome compared to those fetuses who had either a normal or markedly abnormal heart rate tracing in labour (Toomey, Rafferty, & Stamm, 1987).

We have completed a study of the use of antenatal fetal heart rate habituation to predict infant development at 3 years of age. We were also able to assess 32 of these children at 7–8 years of age.

Study Method. The study group consisted of 100 subjects who were tested for habituation less than 2 weeks before their delivery. Fifty-four women had normal uncomplicated pregnancies and 46 had pregnancies complicated by hypertension, intrauterine growth retardation (IUGR), abnormal Dopplers on ultrasound, or developed fetal distress in labour.

Following a 10-min control CTG, the fetus was stimulated for 1 s using a VAS (Corometrics Medical Systems Model 146, Connecticut, USA). This produced an intrauterine sound level of 90 dB (Arulkumaran et al., 1992). The fetal heart rate response was analysed by special computer software that measured the fetal heart rate every 0.2 s. The mean heart for 5 s before the stimulus was compared to the maximum heart rate achieved in the next 55 s after the stimulus. Failure by the fetus to increase its heart rate by more than 10 beat per minute (bpm) for five successive stimuli was regarded as habituation. We counted the number of stimuli required until the fetus reached the habituation criterion. The maximum number of stimuli used was 50. The fetus was classified as a habituator or a non habituator. There were no significant differences in Apgar scores, birth weights, or admission to

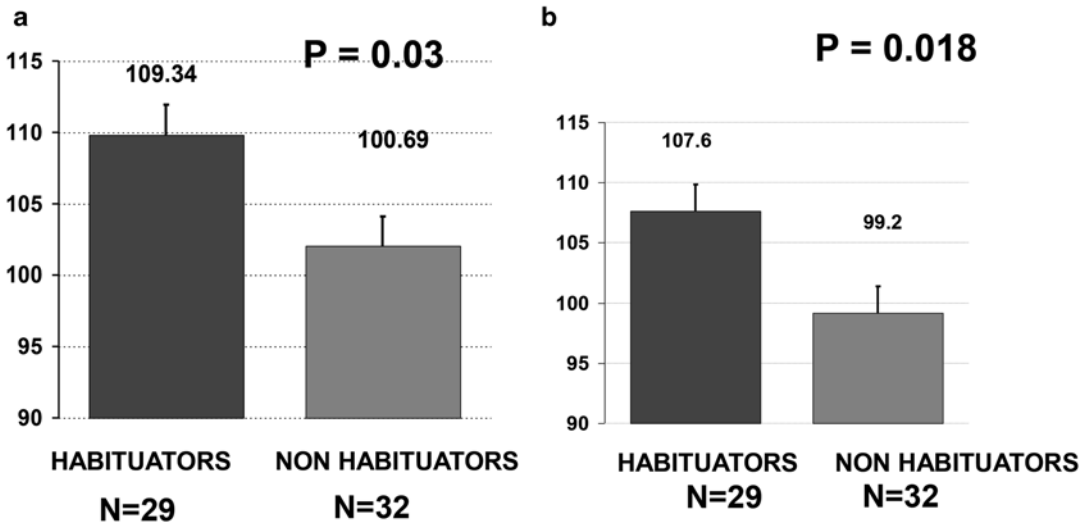

Fig. 11.3 This shows the 36-month Bayley Scales of Infant Development (BSID) scores of the 63 infants who were tested. Panel (**a**) mental developmental index (MDI) and panel (**b**) is the psychomotor developmental index (PDI). Data is presented as the mean ± sd. Fetuses that showed habituation scored significantly higher compared to those who did not ($p < 0.05$)

the special care nursery between fetuses who habituated and those that did not.

Follow-Up. Sixty one infants were tested at 36 months of age by a developmental psychologist who was unaware of the infants habituation status. They were all tested using the Bayley Scales of Infant Development (BSID. Second Edition, 1993). The BSID were used as they are standardized and are the scales most widely used in published research in child development. The fetuses who habituated scored significantly higher on the Mental Development Index (MDI, $p = 0.03$) and the Psychomotor Development Index (PDI, $p = 0.018$) of the Bayley Scales of Infant Development (Fig. 11.3). They scored more highly in the behavior performance ratings. Fetuses who showed heart rate habituation antenatally had a Total Behavioral score (Fig. 11.4) that was significantly higher than those fetuses who failed to habituate ($p = 0.016$).

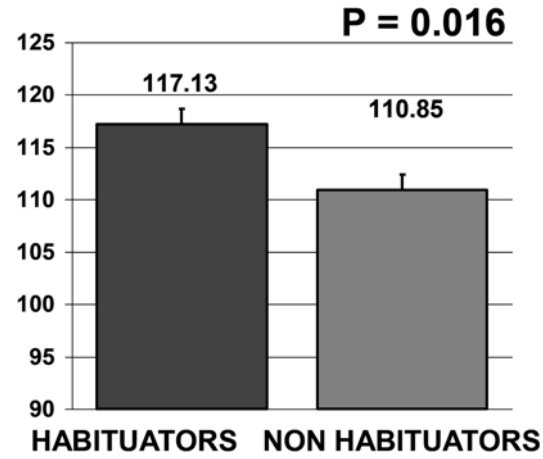

Fig. 11.4 The 36-month Bayley Scales of Infant Development (BSID) for the 63 infants who were tested. Data are presented as the mean ± sd. Behavior Performance Total Score in fetuses who habituated compared to those who did not. Fetuses who habituated scored significantly higher than those that did not ($p < 0.05$)

Fetuses who habituated also performed better in Orientation ($p < 0.009$) and were rated as more confident, co-operative and friendly toward the examiner and took more initiative on the tasks. These same infants were rated more attentive and more able at smoothly shifting their attention from task to examiner and back without losing focus. They were also less hypersensitive and restless and displayed less frustration when doing tasks (Emotional regulation factors, $p = 0.04$).

When the scores were categorized according to being normal (for scores coming within 1 standard deviation of the mean or better) and delayed

Table 11.2 Average child Stanford–Binet Intelligence Scale scores at 7–8 years of age for the groups of fetal habituators and non-habituators shown separately

	All children		Males		Females	
Test	Habit	No habit	Habit	No habit	Habit	No habit
Verbal reasoning	117	106*	115	108.2	118.8	104.5*
Abstr/visual reasoning	113	101.5	108.4	112.6	117.1	93.5
Quantit reasoning	112.6	108	114.4	112.4	111	105.1
Short-term memory	117.9	103.3*	117.5	103.6	118.3	103.1*
Composite score	118	108***	116.4	111.2	119.5	105.7*
Behavioral score total	35.6	27.3*	30.4	30.8	25	39.1**

*$p<0.05$, **$p<0.01$, ***$p=0.051$

(for scores falling 1 or more standard deviations below the mean), the group that showed heart rate habituation displayed significantly better performance compared to the non-habituating group. All six infants who showed a significant delay failed to habituate ($p=0.18$). There were no sex differences in the BSID scores.

Outcome At 7–8 Years. Thirty two children (18 girls and 14 boys) were seen between 84 and 99 months of age. They were assessed by an experienced psychologist using The Stanford-Binet Intelligence Scale: Fourth Edition. This is a standardized test that measures intelligence and cognitive abilities in children and adults, from age two through mature adulthood. Once again the psychologist was unaware of their habituation status.

The results are shown in the Table 11.2 below.

Even though the sample is quite small, those fetuses who habituated had higher scores. The differences tended to be greater in the girls than the boys.

Although the heart rate habituation paradigm we used showed differences in developmental outcome, many infants who failed to habituate had normal developmental scores. Using this method may not be accurate enough to use clinically.

Functional Regression Analysis

In a further study (Ratcliffe, Heller, et al., 2002a; Ratcliffe, Leader, et al., 2002b), we re-analysed the fetal heart rate tracings which had been previously used for the habituation study described above. We did not incorporate any notion of habituation into the model. The aim of the study was to determine if the patterns of heart rate response after VAS generated by functional regression analysis could be used to predict the infant's development at 18 and 36 months of age using BSID.

Method. Fetal heart rate measurements that were made on 73 fetuses using a Corometrics CTG, 14 days or less before labour were reanalyzed. A 1-s VAS (Corometrics) was given every min for a total of 20 stimuli. The heart rate was measured every 0.2 s from 5 s before the first stimulus, until 55 s after the last stimulus. We used new techniques, Functional Data Analysis, to analyse these data (Ratcliffe, Heller, et al., 2002a; Ratcliffe, Leader, et al., 2002b). These methods can be thought of as singular longitudinal data analysis techniques. Standard longitudinal methods could not be used, as there are many more heart rate measurements per fetus than there were fetuses in the study, resulting in singular equations (an infinite number of solutions). Table 11.3 shows the results of the analysis for the MDI of the BSID score at 36 months.

Table 11.3 shows that the sex differences between the groups accounted for 18.8 % of the variance of the BSID whereas the changes associated with VAS accounted for 85.5 % of the BSID variance. Similar results were obtained for the PDI and Total Behavioral Score at 36 months as well. Without stimulation, the heart rates were no better than chance at predicting development. This shows that it is possible to use the changes in fetal heart rate during stimulation to predict the BSID scores.

Table 11.3 Results of the functional data analysis for the mental development index (MDI) of the Bayley scores of infant development (BSID) at 36 months of age

Bayley scales: mental developmental score (MDI) at 36 months					
Linear regression for MDI					
Covariate	*Coef*		*St Dev*	*T*	*p Value*
Constant	10.635		2.939	35.25	0.000
Sex	8.253		3.191	2.586	0.013
R2 = 18.8 %					
Females had a MDI that was 8.25 higher than males. Gender accounted for 18.8 % of the variance					
Functional Regression for MDI					
Covariate	*Coef*		*St Dev*	*T*	*p Value*
Constant	39.411		15.675	2.51	0.020
Sex	13.772		2.036	5.29	0.000
HOME	1.941		0.268	7.25	0.000
ANOVA					
Source	*df*	*SS*	*MS*	*F*	*p Val*
Scalar Cov	3	2437.2	812.4	16.886	0.000
Pulse	24	3545.9	147.7	3.071	0.006
Residual	21	1010.3	48.1		
Total	48	6993.4			
R2 = 85.5 %					
Using stimulated heart rate accounted for 85.5 % of the variance					

Computer Prediction. This form of analysis has enabled us to develop a computer program (Ratcliffe, Leader, Heller, & Dolby, 2000) which automatically imports the heart rate recording at the end of 20 vibroacoustic stimuli and then predicts the infants BSID score at 18 months and 3 years of age. Figure 11.5 shows the data input screen. Knowing the fetal sex improves the accuracy. Figure 11.6 shows the data output screen. We re-analysed 45 fetuses from the original cohort and tested the correlation between their predicted BSID score and their actual score. Data for the MDI at 36 weeks is shown in Fig. 11.7. There is a significant correlation $r = 0.557$, $p = 0.000$ between the infants measured MDI and the computer predicted score. The correlations were also significant for both the PSI ($r = 0.358$ $p = 0.016$) and the Total Behavioral Score ($r = 0.074$, $p = 0.000$).

To test the accuracy of the computer system. FHR responses to repeated VAS were obtained from another sample of 81 pregnant women at 36–38 weeks gestation (Leader et al., 2014). A 1-s VAS (Corometrics) was given every min for a total of 50 stimuli. The BSID scores were assessed at 18 months and 3 years by an independent psychologist. The mean predicted and actual scores were compared. In addition the absolute errors of the predicted scores were compared to corresponding actual scores.

To provide a quantitative value on the error of the predictions, the absolute percentage error of each predicted score was calculated using the formula: [(Actual score-Predicted score)] × 100/ Actual score.

Table 11.4 shows the absolute errors for the BSID scores at 36 months of age. In a small number of cases the errors were very large (>31 %). Excluding these outliers improves the accuracy of prediction.

There is a significant correlation between the actual MDI and the predicted MDI at 36 months (Fig. 11.8, $p = 0.00$).

Functional Data analysis of fetal heart rate changes after VAS shows promise as a tool to predict infant development at 3 years of age. The computer algorithm needs adjustment to try and improve its accuracy at both 18 months of age and 3 years.

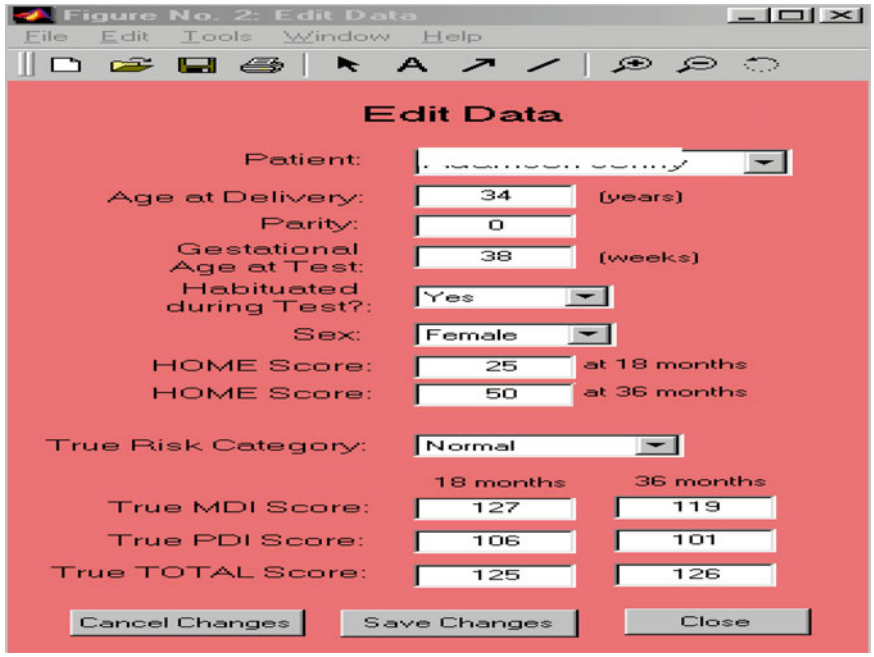

Fig. 11.5 The data entry screen of the software used to predict the Bayley Scales of Infant Development scores from the fetal heart rate response to stimulation

Fig. 11.6 The data results screen of the software showing the predicted Bayley Scales of Infant Development scores from the fetal heart rate response to stimulation

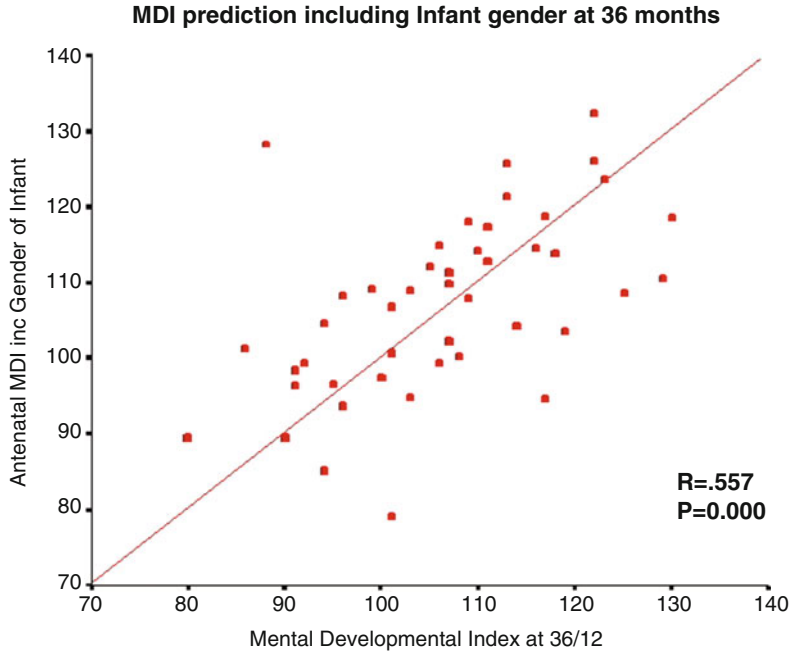

Fig. 11.7 Correlation between the antenatal predicted MDI and actual MDI at 36 months. This includes a correction if the sex of the infant is known ($r=0.557$, $p=0.000$)

Table 11.4 Absolute errors for the predicted Bayley Scales of Infant Development at 36 months of age for the total group and a subgroup with errors less than 31 %

	N	Absolute percentage error (mean ± SD)	*N* (% of Sample)	Absolute percentage error <31 (mean ± SD)
MDI	56	18.3 ± 13.3	51(91 %)	15.1 ± 9.6
PDI	47	18.2 ± 14.9	41(87 %)	14.0 ± 10.6
BRS	59	11.8 ± 8.3	59(100 %)	10.9 ± 7.1

Conclusion

Fetal habituation does provide a measure of CNS function. It is effected by smoking, sedative drugs, decreased O_2 but not significantly by fetal state using vibroacoustic stimuli. There are significant differences between normal and high-risk pregnancies complicated by conditions that are frequently associated with increased neurological damage. It may be possible to identify those fetuses that are at risk and reduce their risk by earlier delivery. The stimulus used in the studies outlined in this chapter did not result in long-term alterations in fetal behavioral state Habituation also can be used as a technique to study the possible effects of drugs such as alcohol, caffeine, and antihypertensive agents in the antenatal period. Habituation can be used to compare pregnancies conceived through the different techniques of assisted conception. Increasing use is being made of preimplantation genetic diagnosis testing (PGD), where one or two cells are removed from an early embryo and tested to ensure that only genetically normal embryos are used for implantation.

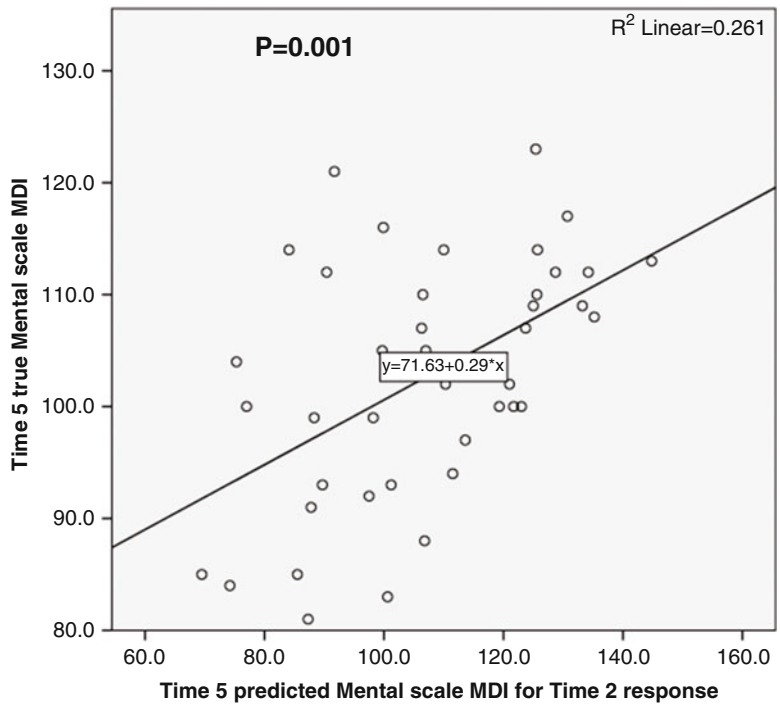

Fig. 11.8 The correlation between the MDI and predicted MDI at 36 months ($p = 0.001$) Absolute error <31

Functional regression analysis is a new tool (Leader et al., 2014) that can be used to analyse fetal heart rate changes after stimulation. If it is possible to predict before birth which infants are at risk of developmental delay, then starting an intervention soon after birth may be able to take advantage of known early neural plasticity. This could prevent a suboptimal outcome rather than the current situation where by the time the diagnosis is made, it is far too late to change that outcome. Any intervention would of course require an appropriate randomized trial to ensure its validity.

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Part III

Environmental Influences on Development: Maternal Emotions

12 Maternal Anxiety, Depression, and Stress During Pregnancy: Effects on the Fetus and the Child, and Underlying Mechanisms

Vivette Glover, Yousra Ahmed-Salim, and Lauren Capron

Abstract

There is good evidence that maternal stress, anxiety, and depression during pregnancy can have long-term effects on a variety of outcomes for the child. We need to understand how an altered emotional state in the pregnant woman affects her biology in a way that in turn affects the development of her fetus. Cortisol is one probable mediating factor, but many other systems also are likely to be important, including the pro-inflammatory cytokines. There is evidence that the function of the placenta is altered if the mother is anxious or depressed and this may control the exposure of the fetal brain to hormones including cortisol, neurotransmitters, and other factors such as brain derived neurotrophic factor that can affect brain development. Epigenetic changes are likely to underlie both changes in placental function and changes in brain structure. We know that most children are not affected by prenatal stress, and that those that are can be affected in different ways. There is evidence that this is, at least in part, because of differential genetic susceptibilities.

Keywords

Prenatal • Stress • Programming • Fetus • Cortisol • Epigenetics • Placenta

Introduction

Both animal and human studies have shown that if the mother is stressed while she is pregnant her child is more likely to have a range of neurodevelopmental problems (Talge, Neal, & Glover, 2007; Van den Bergh, Mulder, Mennes, & Glover, 2005). There is evidence that this association is, at least in part, causal and due to the phenomenon of fetal programming (Glover, 2014). This is the

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concept that the environment in utero, during specific sensitive periods for different outcomes, can affect fetal development with a long-term effect on the phenotype. Fetal programming has been studied especially in relation to fetal growth and later vulnerability to cardiovascular and related diseases (Barker, 2003, 2004). But fetal programming is equally important for the development of psychopathology. Here we will give an overview of the range of effects that prenatal stress can have on the fetus and the child, with a particular focus on neurodevelopment. These have been reviewed extensively elsewhere (Glover, 2014; Talge et al., 2007; Van den Bergh et al., 2005). We also discuss the possible underlying biological mechanisms, including the role of the placenta.

Stress in Pregnancy

Stress is a generic term and has several different definitions. Many different types of prenatal stress have been shown to be associated with altered outcome for the child. These include maternal symptoms of anxiety and depression (O'Connor, Heron, Golding, Beverage, & Glover, 2002), daily hassles (Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2003), pregnancy specific anxiety (Hompes et al., 2013; Huizink et al., 2003) and a poor relationship with the partner (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007). They also include experience of acute disasters such as an earthquake (Glynn, Wadhwa, Dunkel-Schetter, Chicz-Demet, & Sandman, 2001), a Canadian Ice storm (Laplante, Brunet, Schmitz, Ciampi, & King, 2008), a hurricane in Louisiana (Kinney, Miller, Crowley, Huang, & Gerber, 2008), Chernobyl (Huizink et al., 2007), and 9/11 (Yehuda et al., 2005). It is clear that it is not just a diagnosable mental illness or very extreme or "toxic stress" that can alter outcome. Exposures that can have an effect vary from the very severe, such as the death of an older child (Khashan et al., 2008) to quite mild stresses, such as daily hassles. We do not yet know whether different forms of stress have different effects.

Most of these studies are prospective, but those examining the effects of acute disasters are of necessity retrospective, and are less able to allow for possible confounding factors. However, they have the advantage that the effects on the child are more clearly prenatal and unlikely to be due to genetic continuity.

All these studies have shown associations. It is obviously harder to prove that these associations are, at least in part, causal. If the mother is anxious or depressed while pregnant she may be affected postnatally also, and this may affect her parenting. There may be associated factors such as smoking or alcohol consumption, lower maternal education or lower socioeconomic status. These may all affect child outcome. The evidence for thinking that there is a prenatal causal component comes from different types of evidence. First there are animal studies in which the offspring are "cross-fostered" on the first postnatal day, or in the case of primates, reared together in a nursery. These experiments show long-term effects of the prenatal stress, and provide evidence for a prenatal rather than a postnatal or other confounding effect (Weinstock, 2008). Secondly, there are large human studies which have examined associations with prenatal anxiety or depression after allowing for a wide range of potential confounders including paternal mood, postnatal maternal mood, parenting behavior, maternal education, and maternal smoking and drinking alcohol during pregnancy, and still find a clinically significant relationship between prenatal maternal mood and child outcome (O'Donnell, Glover, Barker, & O'Connor, 2014). Thirdly, there are studies which have found associations between prenatal maternal mood and aspects of child outcome at birth, thus showing effects independent of postnatal maternal mood or parenting (e.g., Hompes et al., 2013). And finally, as discussed below, there are studies that are starting to determine possible underlying biological mechanisms, such as changes in placental function.

It has been suggested that mild to moderate stress may actually improve some outcomes. Mild prenatal stress has been shown to be associated with accelerated motor development and cognitive ability for example (DiPietro, Novak, Costigan, Atella, & Reusing, 2006). It is an interesting idea that the pattern of child outcome response to prenatal stress is not linear, with mild

stress causing an acceleration of development and more severe stress an impairment of development. However, other studies have found a linear dose response between prenatal maternal anxiety and emotional/behavioral problems in the child (O'Connor et al., 2002). Thus, it is possible that prenatal stress has different patterns and direction of effect for different outcomes. It would be compatible with this evidence if mild to moderate stress improves physical maturation and cognitive function while also increasing symptoms of anxiety.

Stress also is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis, which produces cortisol (corticosterone in rodents) and the sympathetic adrenal system, which produces adrenaline and noradrenaline. However, the association between maternal psychological symptoms, maternal exposures to stressors, and activation of these biological systems is complex, and can depend on the type and length of exposure. Pregnancy also affects these biological responses, especially those of the HPA axis. Several studies have found little, or a complex association between maternal symptoms of anxiety and depression and cortisol levels during pregnancy (Kane, Dunkel-Schetter, Glynn, Hobel, & Sandman, 2014; Sarkar, Bergman, Fisk, & Glover, 2006). As human pregnancy progresses the placenta produces increasing quantities of corticotrophin releasing hormone (CRH), which in turn stimulates maternal production of cortisol. Cortisol itself stimulates the production of placental CRH by a positive feedback mechanism. By the end of a normal human pregnancy levels of plasma cortisol are double to treble those in the nonpregnant state and as high as those that can be found in depression (Kammerer, Taylor, & Glover, 2006). The HPA axis becomes less responsive to stressors as gestation increases (Kammerer, Adams, von Castelberg, & Glover, 2002), although evening levels (but not morning) have been found to be raised in depressed pregnant women (O'Keane et al., 2011), and pregnant women with material deprivation (Thayer & Kuzawa, 2014). Social support has been shown to buffer increases in HPA axis function during pregnancy (Giesbrecht, Poole, Letourneau, Campbell, & Kaplan, 2013).

Effects on the Child

Many different outcomes have been shown to be changed in association with various types of prenatal stress. The effects can be quite subtle, and often have been measured on continuous scales, rather than by diagnostic categories. However, they also are often of clinical significance (O'Donnell, Glover, Barker, et al., 2014). Here we do not give a comprehensive review of the literature but give selective examples to show the range of outcomes found to be altered. The studies almost all date from the last 10–15 years. Although this type of research has been conducted in animals since the 1950s, it is relatively recently that similar research has been carried out in humans. Some studies have examined large population cohorts such as the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort in Bristol, UK (Glover, O'Connor, Heron, & Golding, 2004; O'Connor et al., 2002, 2007), and used maternal reports of offspring outcome. Other studies have been smaller observational cohorts, such as those of Van den Bergh and her colleagues (Mennes, Van den Bergh, Lagae, & Stiers, 2009; Van den Bergh & Marcoen, 2004) and that of Bergman and colleagues (2007)

The wide range of outcomes which have been found to be altered, include those from birth until adulthood. At birth, an increase in congenital malformations has been found to be associated with very severe stress in the first trimester, such as the death of an older child (Hansen, Lou, & Olsen, 2000). Many studies have shown that less severe stress is associated with somewhat lower birth weight and reduced gestational age (e.g., Rice et al., 2010; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993). Another finding at birth is an altered sex ratio, with fewer males to females being born than in an unstressed population (Obel, Henriksen, Secher, Eskenazi, & Hedegaard, 2007; Peterka, Peterkova, & Likovsky, 2004).

Many studies have looked at neurodevelopmental and psychopathological development. Some investigators have examined the newborn and found a poorer performance on the Neonatal Behavioral Assessment Scale (Field et al., 2003; Rieger et al., 2004), showing that adverse

behavioral outcomes are observable from birth. Studies of infants and toddlers have shown more difficult temperament (e.g., Buitelaar et al., 2003; Davis et al., 2007), more sleep problems (O'Connor et al., 2007), and lower cognitive performance and increased fearfulness (Bergman et al., 2007). More recent studies have shown that effects can be persistent, at least until adolescence and early adulthood (Betts, Williams, Najman, & Alati, 2014; Glasheen et al., 2013; O'Donnell, Glover, Barker, et al., 2014; Pearson et al., 2013).

Others have examined the association between prenatal stress and neurodevelopmental outcomes in children, rather than babies, infants, or adults. Many independent groups have shown increases in child emotional problems, especially anxiety and depression, and symptoms of attention deficit hyperactivity disorder (ADHD) and conduct disorder (e.g., Huizink et al., 2007; O'Connor et al., 2002; O'Connor, Heron, Golding, Glover, & ALSPAC Study Team, 2003; Rice et al., 2010; Rodriguez & Bohlin, 2005; Van den Bergh & Marcoen, 2004), as well as increased vulnerability to victimization (Lereya & Wolke, 2013; Pawlby, Hay, Sharp, Waters, & Pariante, 2011). Others have shown a reduction in cognitive performance (e.g., Laplante et al., 2008; Mennes, Stiers, Lagae, & Van den Bergh, 2006) and differences in learning strategies in adulthood (Schwabe, Bohbot, & Wolf, 2012).

Several studies have found an association between prenatal stress and increased risk of autism or autistic traits (Beversdorf et al., 2005; Kinney et al., 2008; Walder et al., 2014), although one population study failed to replicate this finding (Li, Liu, & Odouli, 2009). Two studies have found an increased risk of schizophrenia in adults. Both showed associations with severe stress, the death of a relative (Khashan et al., 2008) or exposure to extreme adversity, the invasion of the Netherlands in 1940 (Van Os & Selten, 1998), and both showed that the sensitive period of exposure was during the first trimester.

Associations also have been found between prenatal stress and a range of altered physical and physiological outcomes in children. Alterations in brain structure have been observed, reduced brain grey matter density (Buss, Davis, Muftuler, Head, & Sandman, 2010; Sandman, Buss, Head, & Davis, 2014) and altered limbic-prefrontal white matter circuitry (Sarkar et al., 2014). An altered fingerprint pattern (King et al., 2009), and more mixed handedness (Glover et al., 2004; Obel, Hedegaard, Henriksen, Secher, & Olsen, 2003; Rodriguez & Waldenström, 2008) are physical changes observed to be associated with prenatal stress, and are known to be determined in utero. Prenatal stress also has been shown to be associated with an altered diurnal pattern or altered function of the HPA axis, although the pattern of alteration is quite complex (Glover, O'Connor, & O'Donnell, 2010; O'Donnell et al., 2013). Finally, recent studies have shown prenatal stress is associated with reduced telomere length in cord blood cells (Entringer et al., 2013). This is an intriguing finding, as well as of concern, as reduced telomere length is associated with a reduced life span.

Animal studies have consistently found sex differences in the effects of prenatal stress on offspring outcome (e.g., Van den Hove et al., 2013). Female offspring are more vulnerable to increases in anxiety and males to cognitive problems (Weinstock, 2007). In humans, there also is increasing evidence that there may be sex differences in the nature of some of the effects, although the picture is more complex (Glover & Hill, 2012; Glynn & Sandman, 2012; Tibu et al., 2014).

There is little consistency in the literature as to the most sensitive time in gestation for the influence of prenatal stress. It is likely that there are different periods of sensitivity dependent on the outcome studied, and the stage of development of the relevant brain structures. The two studies of schizophrenia found the most sensitive period was in the first trimester (Khashan et al., 2008; Van Os & Selten, 1998). This is when neuronal cells are migrating to their eventual site in brain, a process previously suggested to be disrupted in schizophrenia. In contrast, one study of conduct disorder found the greatest association with stress was in late pregnancy (Rice et al., 2010), as did a study on the risk for autism (Kinney et al., 2008). However, there is inconsistency in the literature even when the same outcome is being considered.

Understanding the times of sensitivity for different outcomes is an area where much more research is needed.

Fetal Stress Responses

There is evidence for continuity from fetal behavior and neurological maturation into early childhood (DiPietro, Bornstein, Hahn, Costigan, & Achy-Brou, 2007). There also is good evidence for an association between the mother's emotional state and the behavior or heart rate of her fetus in later pregnancy (DiPietro, Hilton, Hawkins, Costigan, & Pressman, 2002; Monk, 2001; Van den Bergh et al., 2005). Catherine Monk and her colleagues have conducted studies in which a pregnant mother is asked to carry out a stressful computer task, while the fetal heart rate is monitored (Monk, Myers, Sloan, Ellman, & Fifer, 2003), and shown a correlation between fetal heart rate changes and the mother's self-rated anxiety. Thus, even before birth the fetus can show an immediate response to the maternal emotional state, although we do not know what the mechanism is; the effects are too fast to be due to cortisol, which takes 10–20 min to rise. And noradrenaline does not cross the placenta (Giannakoulopoulos, Teixeira, Fisk, & Glover, 1999).

The fetus can mount its own stress responses from around mid-gestation, independently from any maternal stress response. If a blood transfusion is carried out through the fetal abdomen the fetus shows an increase in β-endorphin and noradrenaline from 18 weeks gestation, and in cortisol from 20 weeks gestation (Giannakoulopoulos et al., 1999; Gitau, Fisk, Teixeira, Cameron, & Glover, 2001).

Underlying Mechanisms

Much remains to be understood about the mechanisms which underlie fetal programming by prenatal stress in humans. One early suggestion was a decrease in blood flow to the fetus (Teixeira, Fisk, & Glover, 1999). However, it is not clear if the decrease observed in that study would be clinically significant, and others have failed to replicate the original finding (Kent, Hughes, Ormerod, Jones, & Thilaganathan, 2002; Mendelson, Dipietro, Costigan, Chen, & Henderson, 2011; Monk et al., 2012).

Another possible mediating factor is increased exposure of the fetus to cortisol (Mina & Reynolds, 2014). Glucocorticoids (cortisol in humans and primates, corticosterone in rodents) are known to have a range of effects on the developing fetus, including on the brain (Herbert et al., 2006). Whilst they are essential for fetal development and tissue maturation, overexposure of the fetus to glucocorticoids may have effects which predispose the child to ill health in later life (Harris & Seckl, 2010). The potentially widespread role for exposure to increased cortisol in human fetal brain development is demonstrated by a study using microarray analysis, which showed that increasing cortisol exposure affected the expression of over a 1000 genes in fetal brain cells (Salaria et al., 2006). A recent study has shown that babies exposed to synthetic glucocorticoids in the womb, because of threatened preterm labour, had more mental health problems than matched controls (Khalife et al., 2013). Davis and colleagues (2013) have shown that babies exposed to synthetic glucocorticoids in utero have altered brain structure, including a thinner cortex, as shown by magnetic resonance imaging scans (Davis, Sandman, Buss, Wing, & Head, 2013). Also, the children of mothers who had consumed high levels of liquorice during pregnancy, (which contains a natural inhibitor of 11β-hydroxysteroid dehydrogenase type II (11β-HSD2), the enzyme which converts cortisol to its inactive form cortisone in the placenta), and were thus exposed to higher levels of cortisol in utero, were more likely to have emotional and cognitive problems in childhood (Räikkönen et al., 2009).

Fetal overexposure to glucocorticoids could occur through increases in maternal cortisol associated with anxiety and during periods of stress, which then crosses the placenta into the fetal environment. In animal models, this has been shown to be one possible mechanism. Administration of adrenocorticotropic hormone

(ACTH) to pregnant rhesus monkeys resulted in increased maternal cortisol production and adverse offspring neurodevelopmental outcomes similar to those seen in response to prenatal stress (Schneider, Moore, Kraemer, Roberts, & DeJesus, 2002). The effects of prenatal stress in rats have been shown to be prevented by adrenalectomy and reinstated by corticosterone administration (Barbazanges, Piazza, Le Moal, & Maccari, 1996). However, the human HPA axis functions differently in pregnancy from most animal models, because of the placental production of CRH, which in turn causes an increase in maternal cortisol. The maternal HPA axis becomes gradually less responsive to stress as pregnancy progresses (Kammerer et al., 2002), and as discussed earlier, there is only a weak, if any, association between maternal mood and her cortisol level, especially later in pregnancy (Kammerer et al., 2006; O'Donnell, O'Connor, & Glover, 2009; O'Keane et al., 2011; Sarkar et al., 2006; Voegtline et al., 2013).

It is possible that fetal programming, caused by prenatal stress, may be mediated by raised fetal exposure to cortisol without increases in maternal levels. Maternal prenatal depression and maternal prenatal cortisol levels have been found to be independent predictors of infant temperament (Davis et al., 2007). Stress or anxiety may cause increased transplacental transfer of maternal cortisol to the fetal compartment without a rise in maternal levels. The placenta clearly plays a crucial role in moderating fetal exposure to maternal factors, and presumably in preparing the fetus for the environment in which it is going to find itself (McKay, 2011; O'Donnell et al., 2009) as part of the predictive adaptive response (Gluckman, Hanson, & Spencer, 2005). Thus, another mechanism by which the fetus could become overexposed to glucocorticoids is through changes in placental function, especially in a down regulation of the enzyme 11β-HSD2, the barrier enzyme which converts cortisol to the inactive cortisone (Togher et al., 2014). If there is less of this barrier enzyme in the placenta then the fetus will be exposed to more maternal cortisol, independently of any change in the maternal cortisol level. If the mother has higher basal levels of cortisol also then the amount of fetal exposure will be higher too, as there is a strong correlation between maternal and fetal cortisol levels (Gitau, Cameron, Fisk, & Glover, 1998). There is evidence in rat models that prenatal stress can affect placental 11β-HSD2. Restraint stress of pregnant rats in the last week of pregnancy has been shown to result in decreased placental 11β-HSD2 expression and activity (Mairesse et al., 2007). Additionally, there is evidence that reduced 11β-HSD2 causes an alteration in the behavior of the offspring. Administration of the 11β-HSD2 inhibitor carbenoxolone in rodent models resulted in an increase in anxiety, mirroring the phenotype of offspring exposed to prenatal stress. Glover, Bergman, Sarkar, and O'Connor (2009) reported that the correlation between maternal and amniotic fluid cortisol levels was greater in women with high anxiety compared to less anxious women. This suggests that prenatal anxiety in humans can increase the placental permeability to cortisol. Our laboratory has found direct evidence that maternal prenatal trait anxiety is associated with a down regulation of 11β-HSD2 in placenta taken from women who underwent an elective caesarean section (O'Donnell et al., 2012).

In utero cortisol has been shown to be inversely correlated with infant cognitive development at about 18 months old (Bergman, Sarkar, Glover, & O'Connor, 2010). This study also measured attachment using the Strange Situation test, and found that this inverse correlation was strong in insecurely attached infants but absent in the securely attached. This suggests that the quality of attachment may be able to buffer against, at least in part, this effect of raised in utero cortisol.

The timing of fetal exposure to raised cortisol also may be important for its effect on cognitive development. Elevated concentrations of maternal cortisol early in gestation has been shown to be associated with a slower rate of development over the first year and lower mental development scores at 12 months of age. However, elevated levels of maternal cortisol late in gestation, were associated with accelerated cognitive development and higher scores at 12 months of age (Davis & Sandman, 2010).

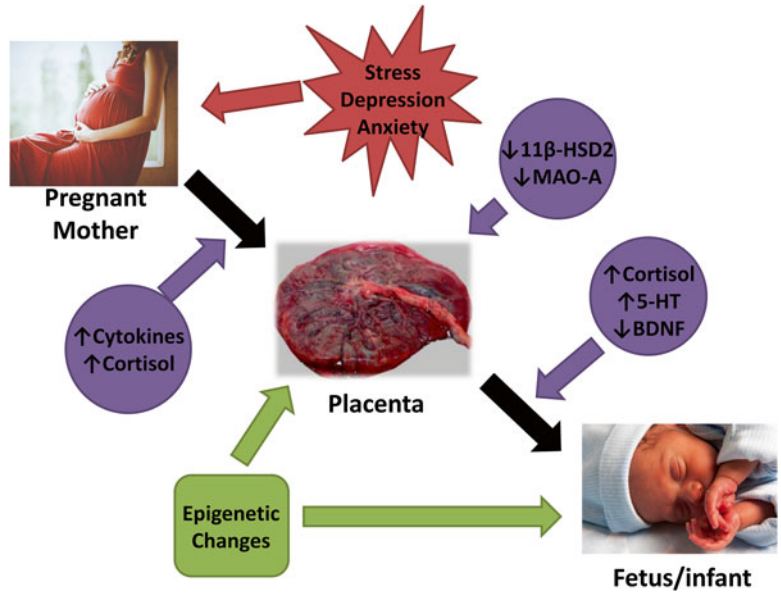

Fig. 12.1 Possible mechanisms by which maternal stress in pregnancy many alter fetal neurodevelopment

Many other systems are likely to be involved as well as the HPA axis and cortisol (Beijers, Buitelaar, & de Weerth, 2014). 5-Hydroxytryptamine (5-HT) is another possible mediator of prenatal stress induced programming effects on offspring neurocognitive and behavioral development. During gestation 5-HT acts as a trophic factor regulating cell division, differentiation and synaptogenesis in the fetal brain (Oberlander, 2012). Animal studies have shown that increased brain 5-HT exposure during gestation is associated with alterations in many neuronal processes and subsequent changes in offspring behavior. Recent work has identified an endogenous 5-HT biosynthetic pathway in the human placenta (Bonnin et al., 2011), suggesting a possible role for alterations in placental 5-HT in human fetal programmming. Prenatal depression has been found to be associated with a down regulation of the expression of placental monoamine oxidase A, the enzyme which metabolizes 5-HT to its inactive metabolite (5-HIAA) (Blakeley et al., 2013). Thus, if the mother is depressed prenatally, the placenta may produce more 5-HT which in turn may result in the brain of her fetus being exposed to more 5-HT, with altered neurodevelopment.

Another potentially interesting mediating factor is brain derived neurotrophic factor (BDNF). This is a trophic factor known to be important in the perinatal period and in synapse formation (e.g., Chikahisa et al., 2006). It is decreased in depression and raised by antidepressants. Prenatal stress in a rat model has been shown to inhibit the formation of mature BDNF in the offspring brain (Yeh, Huang, & Hsu, 2012). A recent study has shown that cord blood levels of BDNF in neonates born to mothers with general anxiety disorder were about half those in controls (Uguz et al., 2013).

Thus, maternal stress, anxiety or depression may be associated with altered placental function, which results in fetal increased exposure to cortisol, 5-HT or decreased BDNF, among other possible factors, and these in turn may change fetal neurodevelopment (Fig. 12.1). These causal pathways all still need to be shown directly. This hypothesis also raises the question as to what biological changes take place in the mother during prenatal stress, anxiety or depression which in turn alters the function of the placenta. As discussed above, the maternal changes in cortisol are not of sufficient magnitude to be a likely sole mediator. And prenatal maternal cortisol levels can be a predictor of child outcome independent of maternal mood (Davis & Sandman, 2010). The maternal mediator, or mediators, between prenatal stress, anxiety and depression and altered child outcome is currently not known.

One possible group of biological maternal mediators could be those associated with the immune system and inflammation, including the pro-inflammatory cytokines, such as interleukin-6 (IL-6) (Buss, Entringer, & Wadhwa, 2013). A mouse model has shown an association between prenatal stress and alterations of immune pathways within the placenta, specifically in male offspring (Bronson & Bale, 2014). Treating the pregnant mice with nonsteroidal anti-inflammatory drugs both reduced the placental cytokine levels and hyperactive locomotion in the male offspring.

There is a growing literature associating pro-inflammatory cytokines with depression in humans (Hepgul, Mondelli, & Pariante, 2010). Increased cytokines have been associated with psychosocial stress during pregnancy (Coussons-Read, Okun, & Nettles, 2007). Elevated maternal stress was related to higher serum IL-6 both in early and late pregnancy. No relationships between stress and cytokines were apparent during the second trimester. However, elevated stress levels across pregnancy were predictive of elevated production of the pro-inflammatory cytokines interleukin-1B and IL-6 by stimulated lymphocytes in the third trimester, suggesting that stress during pregnancy affects the function of immune system cells. A recent study has confirmed that depressed pregnant women have higher levels of IL-6 in the first trimester (Haeri, Baker, & Ruano, 2013). However, another study has failed to find any association between maternal symptoms of anxiety and depression during pregnancy and levels of IL-6 (Blackmore et al., 2011), at 18 or 32 weeks gestation. This is clearly an area that needs further exploration.

Epigenetics

There is currently great interest in the potential role of epigenetics in underlying the long term effects of prenatal stress on the development of the fetus and the child (Monk, Spicer, & Champagne, 2012). Epigenetic changes have been shown to underlie some other developmental origins of vulnerability to disease, such as diabetes (Keating & El-Osta, 2011).

Epigenetics means literally "above" or "on top of" genetics. It is the process through which cells can achieve individuality despite having identical genes. It allows a stem cell to differentiate to form many cell types in embryonic development. The epigenetic modifications to a particular gene control whether it is expressed or silenced and if it is turned on, by how much. These epigenetic changes are heritable in some cases without changes to the genotype. There are many mechanisms by which epigenetic changes can be achieved, and many still remain unknown. Of those we know of, there are direct DNA methylation, chromatin structure modification, histone modification, noncoding RNAs and small RNAs (Gibney & Nolan, 2010). DNA methylation in vertebrates almost always occurs at CpG sites (methylation of the 5′-position of cytosine residues to produce 5-methyl-cytosine). The methyl groups occupy the major groove of DNA, and may disrupt normal recognition of transcription factors and thus suppress expression, or less frequently, enhance expression (Gibney & Nolan, 2010). DNA methylation has been the most studied form of epigenetics in relation to fetal programming.

Several studies have now shown epigenetic changes in the fetus or child after prenatal stress, in both animal models (Jensen Peña, Monk, & Champagne, 2012; Mueller & Bale, 2008; Van den Hove et al., 2013), and in humans (Provençal & Binder, 2014). In a study by Non et al. (2012) investigating genome wide methylation in the umbilical cord blood of neonates exposed to non-medicated maternal depression or anxiety or selective serotonin reuptake inhibitors during pregnancy, compared with unexposed neonates, significantly different DNA methylation levels were found at 42 CpG sites in the neonates exposed to maternal depression or anxiety relative to the controls. Methylation levels were not significantly different between SSRI exposed neonates and controls. Hompes et al. (2013) have shown epigenetic changes in the promoter region of the glucocorticoid (cortisol) receptor in the cord blood from mothers who suffered from pregnancy related anxiety. Oberlander et al. (2008) also have shown epigenetic changes in

this same gene in mothers with prenatal depression and Mulligan, Stees, and Hughes (2012) in newborns of Congolese mothers exposed to extreme psychosocial stress. Teh et al. (2014) have shown the complex relationship between biological inheritance, as represented by genotype and individual prenatal experience and suggest the importance of considering both fixed genetic variation and environmental factors in interpreting epigenetic variation. They found that the best explanation for the variance in the epigenetic pattern in cord blood was the interaction of genotype with different in utero environments, including maternal smoking, maternal BMI, infant birth weight, gestational age, and birth order, as well as maternal depression. All these changes were found at birth, supporting an in utero effect of maternal depression on the epigenome.

Epigenetic changes are likely to underlie some of the placental changes described above, which are associated with prenatal anxiety or depression. For example increased methylation of the placental 11β-HSD2 gene has been shown in the lowest birth weight, healthy, term infants (Wilhelm-Benartzi et al., 2012). Epigenetic changes also may underlie some of the long-lasting effects of maternal adversity, not necessarily mediated by anxiety or depression. Appleton et al. (2013) investigated the association between methylation of the promoter region of 11β-HSD2 in the placenta and markers of prenatal socioeconomic adversity, such as poverty, in 444 healthy newborns. It was found that there was less methylation of 11β-HSD2 in neonates whose mothers experienced the most socioeconomic adversity, and particularly in the male infants.

Epigenetic changes also have been found in older children whose mothers experienced stress during pregnancy. For example maternal prenatal stress, caused by violence by the partner, has been shown to be associated with epigenetic changes in the DNA for the glucocorticoid receptor, in the blood of their adolescent children (Radtke et al., 2012).

Gene/Environment Interactions

A notable finding of all the prenatal stress and child outcome studies is that most of the children are not affected (O'Donnell, Glover, Barker, et al., 2014), and those that are affected can be so in different ways (Bergman et al., 2007). The lack of effect may, in some cases, be due to sensitive postnatal care (Bergman, Sarkar, Glover, & O'Connor, 2008). But it also is probably due, at least in part, to different genetic vulnerabilities, and gene environment interactions (Caspi et al., 2003). Although no interactions have been found between prenatal anxiety, genetic variation in the 5-HT transporter and child outcome (Braithwaite et al., 2013), we are finding interactions between prenatal anxiety and variants of the COMT (unpublished observations) and BDNF genes (O'Donnell, Glover, Holbrook, et al., 2014). If the child has one form of the gene for BDNF, he or she is more sensitive to the effects of prenatal anxiety on internalizing symptoms, but with another form they are less affected. With one form of the gene for COMT cognitive development (working memory assessed by backward digit span recall) is quite sensitive to prenatal anxiety; with another form, there is no effect. A recent study has shown an interaction between prenatal stress and different forms of the D4 dopamine receptor and child outcome. Under conditions of higher prenatal maternal stress, carriers of the DRD4 seven-repeat allele displayed more aggression in adulthood and attenuated cortisol secretion (Buchmann et al., 2014), and also increased antisocial behavior (Zohsel et al., 2014). Homozygous carriers of the DRD4 four-repeat allele were insensitive to the effects of prenatal stress.

All these gene environment interaction effects are quite small and very many different genotypes are likely to be involved. This is certainly an area where further research is warranted, in order to understand more about which children are likely to be affected by prenatal stress, and in what way. Eventually, this research should help in knowing which mothers and children are most likely to benefit from targeted help.

Conclusion

There is good evidence that maternal stress, anxiety and depression during pregnancy can have a long term effect on a variety of outcomes for the child. These effects can be clinically significant and warrant much more effort to provide appropriate intervention and help during pregnancy than is currently the case (Glover, 2014). We have some clues as to potential underlying mechanisms, but much remains to be understood. We need to understand how an altered emotional state in the pregnant woman affects her biology in a way that in turn affects the development of her fetus. Cortisol may play some role but many other factors are also likely to be important. The role of the pro-inflammatory cytokines in pregnancy warrants more investigation. There is evidence that the function of the placenta is altered if the mother is anxious or depressed and that this may control the exposure of the fetal brain to hormones, neurotransmitters, and other factors such as BDNF that can affect brain development. Epigenetic changes are likely to underlie both changes in placental function and changes in brain structure. Finally, we know that most children are not affected by prenatal stress, and that those that are can be affected in different ways. There is evidence that this is, at least in part, because of differential genetic susceptibilities. We need to understand more about these specific vulnerabilities in order to develop the most appropriate interventions.

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Neurobehavioral Consequences of Fetal Exposure to Gestational Stress

13

Curt A. Sandman, Laura M. Glynn, and Elysia Poggi Davis

Abstract

Through intimate communications with the mother, the fetus receives information that is integrated into its neurodevelopmental program to prepare for life after birth. Because the fetal nervous system develops at rapid speed, at precise times and in a specific sequence from conception to birth, disruption in the timing or sequence of development results in tissue remodeling and altered function. Fetal exposures to maternal signals of psychobiological stress are associated with increased risk for behavioral disorders and alterations in brain structures. We have devoted nearly three decades exploring the effects of psychobiological stress in several large cohorts of mothers and their offspring. The focus of this chapter is on the persisting developmental plasticity induced by fetal exposure and adaptation to signals of stress and adversity. Specifically the emphasis is on the emotional, cognitive, and neurological consequences for the newborn, infant, toddler, and child, exposed as fetuses to maternal stress. We review evidence that maternal psychological states and experiences during pregnancy, including stress exposures, mood, fears, and concerns about the course of her pregnancy as well as the level of biological stress signals, exert programming influences on the developing fetus.

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"The most intimate human relationship is between a mother and her fetus" (Haig, 1993, p. 495–496). In addition to the genetic inheritance the fetus obtains nutrients, disposes waste, shares breath and meals with its host. This intimate relationship between mother and fetus includes the reciprocal sharing of cells. Foreign cells cross back and forth through the placenta and invade the fetus (maternal microchimerism) and mother (fetal microchimerism). These cells apparently survive in both mother and the offspring for decades and perhaps forever (Bianchi, Zickwolf, Weil, Sylvester, & DeMaria, 1996; Hall, 2007). Cells present in maternal blood from earlier born children may appear in later born siblings establishing a lineage of plausible influences from mother to fetus (Hall, 2007). There is evidence that these cells migrate to areas of tissue damage (Adams & Nelson, 2004) and are found in many target tissues including the brain (Kaplan & Land, 2005) of the developing fetus. The potential for maternal cells to influence the risk for health outcomes has been suggested (Hall, 2007) but the precise "programming" influence of this intimate relationship between mother and fetus is unknown at this time.

The Fetal Programming Model of Health and Disease is a vivid example to explain the persisting influence conferred by the intimate relationship between a mother and her fetus. This influential model predicts that early or fetal exposures to maternal signals of threat or adverse conditions have lifelong consequences for health outcomes (Barker, 1998). A basic assumption of this model is that developing organisms play a dynamic role in their own construction (Sandman & Davis, 2010; Sandman, Davis, Buss, & Glynn, 2011a, 2011b). A remarkable fetal surveillance and response mechanism has been conserved across species to acquire information about the maternal (or host) environment. The human placenta, a fetal organ, collects information from the maternal environment and responds with a complex series of signals to the host. If the prenatal environment is perceived to be stressful or hostile, the fetal–placental signals to the mother may promote accelerated developmental trajectories, such as preterm birth permitting fetal escape from an inhospitable environment and ensuring short-term survival. The fetus also incorporates this bidirectional information to adjust its developmental program in preparation for survival after birth. The information may cause (or program) the fetus to modify its nervous system to adapt to an expected postpartum environment.

Why study the human fetus? Because the fetal period in the life cycle is unmatched by any other in growth and development; it is the stage in the human life span that is most vulnerable to both organizing and disorganizing (programming) influences. Tissues develop in a specific sequence from conception to maturity making different organs sensitive to environmental influences at different times. Critical periods are epochs of rapid cell division and it is during these periods that organs are especially vulnerable to perturbations such as stress. Thus, the timing of the stimulus during development coupled with the time-table for organogenesis determines the nature of a programmed effect. A variety of maternal stressors can retard fetal growth and result in damage to vital organs. Regardless of the developmental timetable for organ development, compensations are made by the fetus to protect the brain. For instance, maternal adversity may decrease the blood supply available to the fetus. To protect the brain, blood is shunted from peripheral organs to nurture the fetal nervous sys-

tem. This adaptation may result in damage to peripheral organs, and although the nervous system experiences less deprivation to oxygen and nutrients, this offers only partial protection because the vulnerable fetal brain cannot escape the circulating biochemical fingerprint of maternal stress. The human fetal nervous system is a primary target for these circulating programming influences because it is undergoing dramatic growth over a prolonged period of time. As reviewed by Stiles and Jernigan (2010), neuron production in humans begins on embryonic day 42 and is complete by midgestation. Between gestational age (GA) 8 and 16 weeks, migrating neurons form the subplate zone awaiting connections from afferent neurons originating in the thalamus, basal forebrain, and brainstem. Concurrently, cells accumulating in the outer cerebral wall form the cortical plate which eventually will become the cerebral cortex. By gestational week 20, axons form synapses with the cortical plate and by gestational week 24, cortical circuits are organized (Bourgeois, Goldman-Rakic, & Rakic, 1994; Kostovic, Judas, Rados, & Hrabac, 2002). By gestational week 28, the human brain contains billions of neurons and is 40 % greater than in the adult (Becker, Armstrong, Chan, & Wood, 1984; Bourgeois et al., 1994; Huttenlocher & Dabholkar, 1997; Huttenlocher, de Courten, Garey, & Van der Loos, 1982). The rate of synaptogensis reaches an astonishing peak so that at gestational week 34 through 24 months postpartum, there is an increase of 40,000 synapses per minute (Levitt, 2003).

The focus of this chapter is on the persisting developmental plasticity induced by fetal exposure and adaptation to signals of stress and adversity. Specifically the emphasis of this chapter is on the emotional, cognitive, and neurological consequences for the newborn, infant, toddler, and child, exposed as fetuses to maternal stress. We review evidence, especially from our own research program, that maternal psychological states and experiences during pregnancy, including stress exposures, mood, fears, and concerns about the course of her pregnancy as well as the level of biological stress signals, exert programming influences on the developing fetus.

Stress

In physics, stress is defined as the degree of distortion in a malleable metal when it is subjected to an external load. It is the ratio of force applied, to the area affected. Selye adapted this physical definition of stress for the life sciences as the "nonspecific response of the body to any demand for change" (Selye, 1936, p. 32). He emphasized the non-specificity of stressful events—they could be heat, cold, exercise, bacterial infection, and a host of other agents (Selye, 1959). Selye broadened his definition over the years by adding to the concept the idea that stress included an inadequate physiological response to any demand that resulted in "wear and tear on the body" (Selye, 1956). His General Adaptation Syndrome acknowledged that individuals adapted to, and developed defenses against stress (Selye, 1955).

Biological Stress System

Modern biological models of stress emphasize the importance of one of the major stress systems, the hypothalamic–pituitary–adrenal (HPA) system, for adaptation and disease. Systemic stress activates the expression of the master stress hypothalamic hormone, corticotrophin releasing hormone (CRH), which stimulates the cascade of events preparing the organism for "fight or flight." The neuropeptide, CRH, is synthesized primarily in the paraventricular nucleus of the hypothalamus and has a major role in regulating pituitary and adrenal function and the physiological response to stress (Chrousos, 1992; Vale, Spiess, Rivier, & Rivier, 1981). CRH stimulates the synthesis of a bioinactive prohormone, proopiomelanocortin (POMC) in the anterior pituitary gland which is converted by enzymes into adrenocorticotrophic hormone (ACTH), and other active peptides. ACTH enters the blood stream and elicits secretion of glucocorticoids (cortisol in humans) from the zona fasciculata in the adrenal gland. The acute response to ACTH results in an increase in the supply of cholesterol (the substrate for glucocorticoids/cortisol) to steroidogenic enzymes resulting in

the liberation of cortisol. The negative feedback among the adrenal gland, the pituitary gland, and the hypothalamus terminates the stress response under normal or acute conditions. The chronic (hours to days) adrenal response to ACTH results in an increase in the transcription of the genes that encode steroidogenic enzymes (increasing cortisol levels) resulting in failure of the negative feedback loop and increased risk for stress-related disease. In addition, cortisol crosses the blood–brain barrier and activates specific receptors in limbic and cortical brain structures. The limbic structures, especially the hippocampus, prefrontal cortex (PFC), and amygdala, influence the negative feedback system by their excitatory and inhibitory connections with HPA axis (Avishai-Eliner, Brunson, Sandman, & Baram, 2002).

Endocrine Changes in the Stress System During Pregnancy

The maternal "fight or flight" endocrine, immune and vascular systems are profoundly altered during the course of human pregnancy (de Weerth & Buitelaar, 2005; Entringer et al., 2009; Glynn, Dunkel-Schetter, Hobel, & Sandman, 2008; Mairesse et al., 2007; Sandman & Davis, 2012; Sandman, Davis, & Glynn, 2012a) presenting the fetus with continuous and escalating levels of "information." For instance, the pituitary gland doubles in size increasing by several fold the synthesis, and release of pituitary peptides into the maternal circulation. Production from target tissues, such as cortisol from the adrenal gland also increases over the course of pregnancy. But it is the growth and development of a new fetal organ, the placenta in primates that is primarily responsible for the profound changes in the maternal/fetal stress systems.

The human placenta synthesizes and releases CRH into the maternal and fetal circulation. Placental CRH is identical to hypothalamic CRH in structure, immunoreactivity and bioactivity (Petraglia, Sutton, & Vale, 1989; Sasaki et al., 1987, 1988). However, in contrast to the inhibitory influence on the promoter region of the CRH gene in the hypothalamus, maternal stress signals (cortisol) from the adrenal glands activate the promoter region in the placenta and stimulate the expression of hCRHmRNA establishing a positive feedback loop that allows for the simultaneous increase of placental CRH (pCRH), ACTH and cortisol over the course of gestation. All of the stress related peptide/hormone levels rise as pregnancy advances (see Fig. 13.1), peaking during labor, and falling to basal or, in the case of pCRH, undetectable levels within 24 h after delivery (Campbell et al., 1987; Chan et al., 1993; Goland, Conwell, Warren, & Wardlaw, 1992; Sasaki et al., 1987; Wolfe et al., 1988). The exponential increase of pCRH especially over the latter part of human gestation plays a fundamental role in the organization of the fetal nervous system (Sandman, Wadhwa, Chicz-DeMet, Porto, & Garite, 1999): it plays a key role in the maturation of the fetal HPA axis and other systems, influencing the timing of the onset of spontaneous labor and delivery (McLean et al., 1995; Sandman et al., 2006; Smith, Mesiano, & McGrath, 2002; Smith & Nicholson, 2007; Tyson, Smith, & Read, 2009) and in maternal adaptation during pregnancy, including dampening psychological stress (Glynn & Sandman, 2011; Sandman, Davis et al., 2011a).

Concurrent with increases in circulating levels of pCRH, a binding protein (CRH-BP) is produced in the liver and also in the trophoblast and intrauterine tissues during pregnancy, and binds to circulating CRH, reducing its biological action (Orth & Mount, 1987; Petraglia et al., 1993; Petraglia, Florio, Nappi, & Genazzani, 1996). In contrast to the exponentially increasing levels of circulating pCRH over the course of gestation, CRH-BP levels, which are constant in the first, second, and early third trimester and are not significantly different from nonpregnant levels, fall by approximately 30 % as birth approaches (Linton et al., 1993). The net effect of these changes in levels of pCRH and CRH-BP is a sharp increase in the availability of free and bioactive CRH during this last part of gestation.

Maternal plasma cortisol binding globulin (CBG) levels also change across pregnancy. CBG levels stimulated by estrogen increase progressively with advancing gestation until the end of

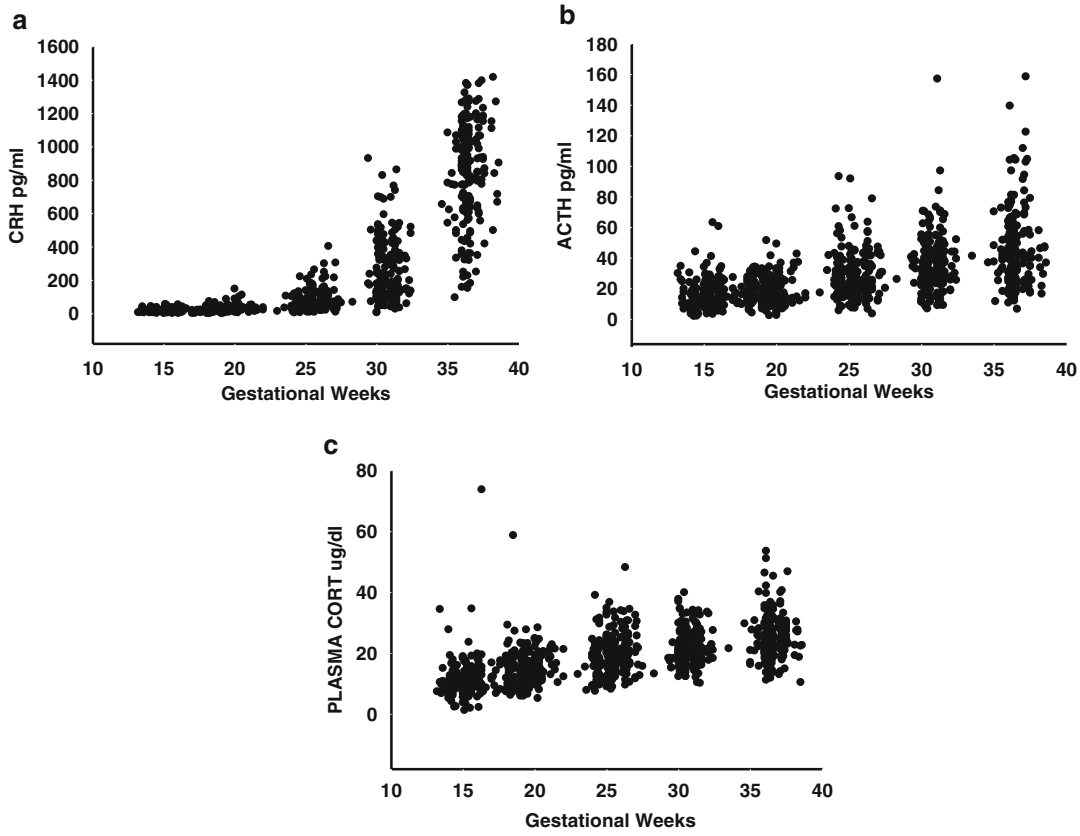

Fig. 13.1 Maternal plasma levels of pCRH (**a**), ACTH (**b**), and Cortisol (**c**) increase significantly as gestation progresses. Levels of pCRH increase as much as 40-fold and ACTH and cortisol levels increase two- to threefold as term approaches

gestation when there is a significant decline leading to an increase in bioactive cortisol (Ho et al., 2007). The activity of a placental enzyme, 11β-HSD2 (which oxidizes cortisol into its inactive form, cortisone) (Sun, Adamson, Yang, & Challis, 1999), increases as gestation progresses before falling near term. Both the decrease in CBG and the decrease in activity of placental 11β-HSD2 as pregnancy advances serve to increase fetal exposure to maternal cortisol ensuring maturation of the fetal lungs, the brain, and other organ systems in full-term births (Ma, Wu, & Nathanielsz, 2003; Murphy & Clifton, 2003).

In addition to these profound but normal changes in basal endocrine levels during pregnancy, the human placenta integrates numerous sources of maternal stress signals and responds with a dose dependent release of CRH. Detection by the fetal–placental unit of stress signals from the maternal environment (for instance, cortisol) "informs" the fetus that there may be a threat to survival. The placental/fetal unit responds to this by synthesizing and releasing CRH (positive feedback) and activating a cascade of consequences. One maternal consequence is myometrial activation and depending on the timing during gestation and the severity of the stress, could result in abbreviated gestation and early fetal escape from a hostile environment. In parallel, the fetus adjusts its developmental trajectory and modifies its nervous system to ensure survival in a potentially hostile postpartum environment. Survival under these conditions can be associated with compromised growth, reproductive success, motor, cognitive and emotional function.

Psychological Stress

Currently, no consensus exists regarding a unifying definition of psychological stress or the best way in which to measure it during the perinatal period. The theoretical perspective employed in our work is consistent with the view provided in a classical volume on stress in which Appley and Trumbull (1967) identified three components: (1) The "stimulus side" which consists of "new, intense, rapidly changing, sudden or unexpected" situations that approach the upper limits of tolerance. (2) The "response side", is defined as the presence of "emotional activity" from which stress may be inferred. States such as anxiety or depression or any behavior that deviates from normal are examples that could be the result of stress. (3) The existence of a stress state "within" an individual determined from physiological or biological responses such as changes in the autonomic nervous or endocrine systems similar to those described above. Our conceptual approach also has been heavily influenced by the work of Richard Lazarus (1966, 1968), which also characterizes stress as consisting of stimulus events or stressors (environmental exposures) and responses (these include biological, emotional, cognitive and behavioral reactions). An additional theoretical component we incorporate from the work of Lazarus is cognitive appraisals of stress, which operate as critical intermediaries between stressors and responses (Lazarus & Folkman, 1984).

In addition to the difficulty of defining the complex concept of stress more generally, pregnancy presents a further unique challenge because psychological stress responding and mood states change reliably as pregnancy progresses. For example, we have documented that as gestation advances, women become less psychologically responsive to stressors in the environment, exhibiting alterations in stress appraisals (Glynn et al., 2008; Glynn, Dunkel-Schetter, Wadhwa, & Sandman, 2004; Glynn, Wadhwa, Dunkel-Schetter, Chicz-DeMet, & Sandman, 2001). For instance, women rated their response to a major earthquake as more stressful when it occurred early in pregnancy compared with women who were exposed to the same event later in pregnancy (Glynn et al., 2001). Further, during pregnancy there are predictable changes in generalized anxiety symptoms (Glynn et al., 2008), fears and anxieties specific to pregnancy and in depressive symptoms (see Fig. 13.2). Because the experience and manifestations of stress during pregnancy are dynamic and because no clear agreement exists regarding the optimal way in which to measure it, we have taken a longitudinal and multidimensional approach in our studies. These methodologies consist of repeated assessments (usually 4–5 during gestation) and the measurement of stressful stimuli (e.g., life event exposures), stress appraisals (e.g., perceived stress), responses (e.g., anxiety and depressed mood), and challenges unique to pregnancy (e.g., pregnancy-specific anxiety).

We have devoted two decades studying several large cohorts of mothers and their offspring, and we have identified two particularly potent prenatal predictors of infant and child outcomes: anxiety specific to pregnancy and depressive symptomatology. We have found that pregnancy-specific anxiety (PSA), a measure developed in our laboratory (see Table 13.1 for PSA scale questions) which assesses a woman's feelings about her health during pregnancy, the health of her baby, and her feelings about labor and delivery, is linked to birth outcomes, infant HPA, emotional and cognitive and child emotional regulation, cognitive functioning, and brain development. Further, maternal depressive symptoms during pregnancy have been linked to infant emotional regulation and child behavioral regulation and brain development. In our view the consistent findings associated with these constructs which have emerged from among those comprising our multidimensional approach, represent important steps forward regarding theoretical specificity of definitions of prenatal stress relevant to predicting cognitive and emotional child outcomes.

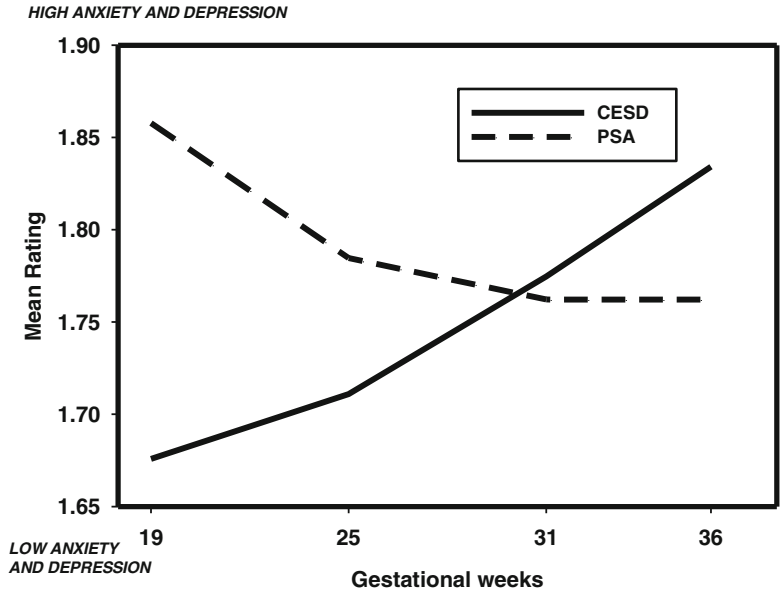

Fig. 13.2 Among our healthy participants ($N = 536–762$), prenatal reports of depressive symptoms (assessed with the Centers for Epidemiologic Studies Depression Scale, CESD) ($F_{(3, 1409.2)} = 17.53$, $p < 0.001$; Greenhouse–Geisser) increase over the course of gestation. The increase in depressive symptoms as gestation progresses stands in contrast to the decrease ($F_{3, 1537.5} = 19.27$, $p < 0.001$; Greenhouse–Geisser) in pregnancy-specific anxiety (PSA) across gestation. It is obvious that the slopes across gestation of these two assessments of maternal mood are significantly different ($F_{1,629} = 50.78$, $p < 0.001$) and suggests that even though there are modest (but significant) correlations between them at specific gestational intervals (r's range from 0.30 to 0.40) they are sensitive to unique parameters of maternal mood as pregnancy advances. The scores are the average of a Likert Scale, 4-point ratings for both CESD and PSA

Table 13.1 Scale to assess fears and concerns about pregnancy

Pregnancy specific anxiety (PSA)
1. I am confident about having a normal childbirth
2. I think my labor and delivery will go normally
3. I am fearful regarding the health of my baby
4. I am worried that the baby might not be normal
5. I am afraid that I will be harmed during delivery
6. I am concerned or worried about how the baby is growing and developing inside me
7. I am concerned or worried about losing the baby
8. I am concerned or worried about having a hard or difficult labor and delivery
9. I am concerned or worried about taking care of a new baby
10. I am concerned or worried about developing medical problems during the pregnancy

Each item is scored on a four point scale from 1 (never or not at all) to 4 (almost all of the time or very much)

The reliable ($r = 0.75–0.85$) 10-item PSA was developed in our laboratory to assess a woman's feelings about her health during pregnancy, the health of her baby, and her feelings about labor and delivery (Glynn et al., 2008). Answers are given on a 4-point scale. This scale has been used in studies of preterm birth and child outcome (Blair et al., 2011; Buss et al., 2010; Buss et al., 2011; Davis et al., 2004, 2007; Davis, Buss et al., 2011; Davis & Sandman, 2010; Glynn et al., 2008; Sandman, Davis et al., 2011a)

Psychological and Biological Stress During Pregnancy

A fundamental assumption of our work is that maternal HPA and placental hormones represent primary mechanisms underlying the effects of maternal psychological distress on infant and child development. Support for this assumption requires that there are associations between biological and psychological stress measures. However, in the existing literature to date, there is minimal to no support for these associations during human pregnancy. More often than not, we and others do not detect significant associations between biological and psychological stress indicators at any point in gestation. Moreover, studies by us and others that have included both psychological and biological indicators of stress have found that they often are independent predictors of child outcomes. New findings suggest that the limited evidence for an association may be due, at least in part, to the methodologies (both in study design and in analysis strategies) typically employed. For example, when maternal signals were measured repeatedly over the course of the day, maternal psychosocial state and biological signals were marginally but significantly associated (Entringer, Buss, Andersen, Chicz-De Met, & Wadhwa, 2011).

Another possible and perhaps stronger approach for detecting associations between psychological and biological signals is to examine trajectories or profiles across time rather than single time points. We recently showed in a sample of 448 women, that those with high levels of pregnancy specific anxiety during pregnancy had the steepest increases in the trajectories of cortisol over gestation (Kane, Dunkel-Schetter, Glynn, Hobel, & Sandman, 2014). At present, there are no firm conclusions about the associations between biological and psychological stress during pregnancy. The suggestions that refined temporal resolution or consideration of patterns of change as pregnancy advances improves the ability to detect significant associations between these two domains of stress must be balanced with the evidence that there is minimal or no association at single time points during gestation and that when these two sources of stress predict the same outcome, their influence is usually independent. Moreover, it is important to acknowledge that the large majority of conclusions about the association between biological and psychological stress are based on studies that have relied heavily on measures of cortisol. It is possible that other peptides and hormones with different temporal patterns, half-lives, circadian dependencies, and rates of synthesis and release have unique and perhaps significant associations with psychological measures of stress.

Rationale for Our Outcome Measures

The genesis of the fetal programming model was the significant association observed (retrospectively) between adult health outcomes and birth phenotype (gestational age at birth and birth weight). In our studies, we assess birth phenotype both as a measure of health outcome associated with fetal exposures and as a predictor (and covariate) of subsequent developmental outcome. Our neurobehavioral studies of the fetus have included measures of heart rate and movement patterns during resting states, responses to startling stimulation and rates of habituation to repeated stimulation. In the neonate we have examined neurological development and integrity of the HPA axis as a consequence of fetal exposures to maternal signals.

Fetal programming studies in infants and children have been focused primarily on outcome measures of emotional adjustment and cognitive ability. We selected measures that are psychometrically sound and stable across a broad range of development. Stability of outcome increases the probability that associations discovered between fetal exposures and early life outcomes will be significant markers of risk later in development. The primary measures of infant and toddler emotional adjustment are age-appropriate subscales of the Infant, Toddler, Child Behavior Questionnaire (Gartstein & Rothbart, 2003). Emotional adjustment in preadolescent children is assessed with the Child Behavior Check List

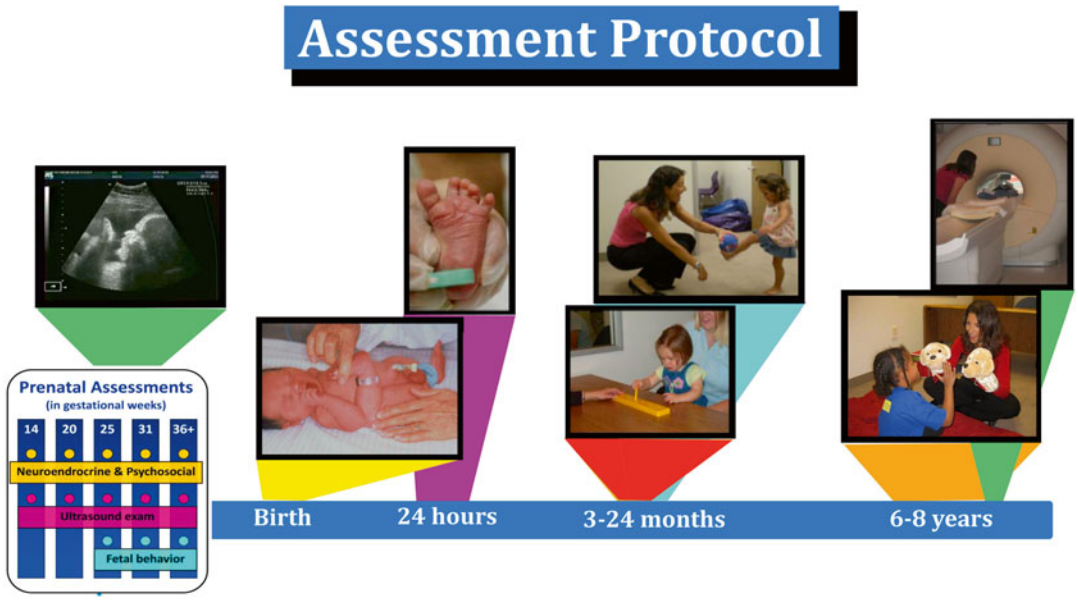

Fig. 13.3 The general assessment protocol and timeline of our longitudinal project of the consequences of fetal experience on child development, beginning at ~15 weeks of gestation and continuing through early adolescence

(CBCL), one of the most widely used instruments in both research and clinical practice with children (Achenbach & Rescorla, 2001). Measures of recognition memory, habituation, visual preference and simple problem solving are obtained with the Mental Developmental Index (MDI) of the validated Bayley Scales of Infant Development (Bayley, 1993). The Psychomotor Development Index (PDI) assesses quality of infant movement, sensory integration and perceptual-motor integration including fine motor development. After 5 years of age, cognitive ability is assessed in two ways: (1) a battery of computerized neuropsychological tasks and (2) with WISC IV (Perceptual Reasoning Scale) and the WRMAL (Memory) (Sheslow & Adams, 2003; Wechsler, 2002). The specific assessments of emotional adjustment and cognitive abilities are not identical at different developmental intervals but are age-appropriate for the fetus, neonate, infant, and child.

In our preadolescent cohort we perform structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) scans to determine if there are distinctive neurological patterns associated with fetal exposure to psychobiological stress. Moreover, we test mediational models to examine the relations among predictors, outcomes, and neurological patterns. Figure 13.3 presents our typical protocol and timeline for assessments of the fetus, infant, and child.

Prenatal Stress and Birth Phenotype

The earliest and strongest evidence in support of the fetal programming of health and disease across the lifespan was from retrospective studies on birth outcomes. These pioneering studies indicated that being born small for gestational age or being born early was associated with increased risk for numerous poor health outcomes such as cardiovascular disease, hypertension, hyperlipidemia, insulin resistance, non-insulin dependent diabetes mellitus, obesity, higher serum cholesterol concentrations, and shortened life span (Barker, 1998; Barker, Osmond, Simmonds, & Wield, 1993; McCormack et al., 2003; Richards, Hardy, Kuh, & Wadsworth, 2001; Roseboom et al., 2000). These retrospective studies provided compelling evidence for fetal programming.

But they could not tell us why the fetus was born small or early. Recent research from our laboratory and others have evaluated whether psychobiological stress contributes to birth phenotype.

Biological markers of stress consistently have been associated with adverse birth outcomes. The findings for psychosocial stress have been less consistent. The general findings among methodologically sound studies are that women reporting elevated levels of psychosocial stress during pregnancy are at significant risk for adverse birth outcomes (Dunkel-Schetter & Glynn, 2011; Lobel, 1994; Paarlberg, Vingerhoets, Passchier, Dekker, & Van Geijn, 1995). Reports from our group (Campos et al., 2008; Dominguez, Dunkel-Schetter, Glynn, Hobel, & Sandman, 2008; Glynn et al., 2001, 2008; Hilmert et al., 2008; Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1999; Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993) that have accounted for the effects of potential confounders (including medical risk, parity, substance use, etc.) revealed significant associations between prenatal stress and adverse birth outcomes. Moreover, many studies have reported that elevated stress "early" in gestation is associated with the greatest risk for adverse birth outcomes. Consistent with the assertion that timing of stress exposures is a critical determinant of the association between stress and birth outcome, we (Glynn et al., 2008) reported in a large ($N=418$) longitudinal study of pregnancy that the *pattern* of stress and anxiety during pregnancy was a better predictor of birth outcomes than stress or anxiety assessed at any one point during pregnancy. Further, as we presented in Fig. 13.2, maternal reports of pregnancy-specific anxiety typically decrease as pregnancy advances. These findings that patterns of stress across gestation contribute to outcomes as well as those examining specific timing effects during pregnancy are strong arguments for longitudinal studies.

Within the stress pathway, pCRH is most strongly linked to gestational length. Placental CRH has been characterized as controlling a "placental clock" that determines or alters the timing of onset of parturition (McLean et al., 1995; Smith et al., 2002). Elevated levels and steeper trajectories of pCRH initiate a cascade of events resulting in myometrial activation and in extreme cases preterm birth (Hobel, 2004; Hobel, Arora, & Korst, 1999; McGrath et al., 2002; Smith et al., 2009; Wadhwa et al., 2004; Wadhwa, Porto, Garite, Chicz-DeMet, & Sandman, 1998). Importantly, it is the trajectory of pCRH production (i.e., the rate of acceleration), rather than the absolute hormone concentration that best predicts preterm birth suggesting that target cells are highly responsive to relative changes in pCRH concentrations (Smith et al., 2009).

In a study to examine the association between birth outcome and a panel of biological stress markers, maternal levels of B-endorphin, ACTH, cortisol and pCRH were assessed at regular intervals from 15 to 36 weeks of gestation in 203 pregnant women (Sandman et al., 2006). Consistent with previous studies, pCRH levels in women destined to deliver preterm (before 37 weeks) had faster rates of increase and significantly higher levels of pCRH confined to the beginning of the early third trimester than women who subsequently delivered at term. Of the other maternal measures, only cortisol, as early as 15 weeks of gestation, was elevated in women delivering preterm. Models that accounted for the independent and shared variance of pCRH and cortisol indicated that only pCRH between 26 and 31 weeks of gestation predicted gestational length. However, we found that the best predictor of pCRH at 31 weeks was maternal cortisol at 15 weeks. The findings from this study indicated that a plausible stress-related endocrine signal, elevated cortisol from the mother very early in pregnancy predicts the precocious rise in CRH leading to an abbreviated gestation. The pattern of findings supports the argument that the effect of elevated cortisol early in pregnancy reflects priming or programming (Barker, 1998; McLean et al., 1995; McLean & Smith, 2001) effects on the eventual fetal–placental CRH response.

In addition to the regulation of gestational length, pCRH plays a key role in the regulation of fetal maturation with consequences for birth phenotype. Neonatal evaluations of neuromuscular

and physical characteristics of the newborn that develop over the course of gestation (Ballard et al., 1991) often are done within 24 h of birth to assess developmental maturation. In a study from our group (Ellman et al., 2008) of 158 newborns within 24 h after birth we found that fetal exposure to increased levels of maternal cortisol at 15 and 19 weeks of gestation and increased levels of pCRH at 31 weeks of gestation were associated with significant decreases in newborn physical and neuromuscular maturation. These effects were observed after adjusting for length of gestation.

Women with signs of preterm labor often are administered a course of treatment with betamethasone (a synthetic glucocorticoid) to ensure maturation of the fetal lungs and to maximize infant viability. Many infants exposed to this treatment are born term so that the effects of exposure can be determined without the complications associated with abbreviated gestation. We (Davis et al., 2009) assessed the effects of fetal exposure to betamethasone on size at birth among 105 term infants. The importance of this approach for this discussion is that synthetic glucocorticoids are not bound or oxidized so they freely pass the placental barrier to the fetus. Compared to matched controls, treated infants were shorter, weighed less and had reduced head circumference at birth after adjusting for fetal size. This approach and the findings complement our studies of naturally occurring cortisol and support the conclusion that fetal exposure to glucocorticoids has a direct and significant influence on human fetal development.

Prenatal Stress and Fetal Behavior

"The focus of fetal neurobehavioral development research is to gather information on the functional development of the fetus with the putative expectation that function provides meaningful representation of nervous system development" (DiPietro, Costigan, & Voegtline, 2015). DiPietro et al. (2015) argue that studies of the fetus provide information on the ontogeny of neural maturation and on factors that might disrupt normal development. An additional advantage of assessment of fetal behavior is that consequences of intrauterine exposures can be evaluated before there are postpartum influences. However, there are limitations to what can be achieved because of the narrow range of fetal behavior that can be measured, the obvious issue of access to the fetus and the related issue of ethical constraints. Measures of fetal responses to mild external stimulation (vibroacoustic and acoustic) have been used in our projects (Sandman, Wadhwa, Hetrick, Porto, & Peeke, 1997) to assess directly the developmental consequences of exposure to endocrine markers of stress ("disruptors" of normal development) (Sandman, 2010).

We have considered the influence of fetal exposure to maternal and placental hormones on fetal development. As discussed above, we particularly have been interested in pCRH because it can be viewed as evidence that the fetus is responding to maternal stress. In a prospective longitudinal study, we assessed fetal nervous system maturation at 25, 31, and 37 weeks of gestation by monitoring fetal heart rate (FHR) responses to vibroacoustic stimulation (VAS)[1] in 191 maternal–fetal dyads (Buss et al., 2009). Reliable startle responses were not detected in all fetuses until 31 weeks gestational age. Because there was evidence that some fetuses did respond at 25 weeks, we wondered if there were individual differences that could be explained by exposures to placental markers of stress. To examine this we (Class et al., 2008) evaluated in 138 maternal–fetal dyads the association between startle responses at 25 weeks and pCRH levels collected at 15, 20, and 25 weeks of gestation. Placental CRH levels at 15 weeks of gestation, but not later points, predicted enhanced FHR responses to the VAS. These findings were interpreted to suggest that fetal exposure to the lowest concentrations of pCRH (optimal, low stress exposure) early in gestation reflected greater fetal maturity and

[1] The device and administration influences the results (Kisilevsky, Fearon, & Muir 1998). On the mother's abdomen we applied a 1 s administration of the VAS (72 dB, 75 Hz + 10 % harmonics ranging from 20 to 9000 Hz; EAL Model 146, Corometric Medical System, CT, USA).

accelerated neurological development. Similarly, in an earlier study we reported that fetal exposure to pCRH later in gestation also exerts programming effects on the fetus. Lower placental CRH during the third trimester was associated with an improved ability to habituate to repeated presentations of the same stimulus and to identify a novel stimulus (related to attention and memory) (Sandman et al., 1999). These findings suggest that not only is pCRH related to birth outcomes and timing of delivery but also to fetal maturation.

Placental CRH as a fetal signal may play a key role in the regulation of fetal maturation. We additionally have evidence that maternal adrenal and pituitary hormones/peptides influence fetal developmental trajectories. Few studies have evaluated the implications of maternal cortisol on fetal behavior. In a recent study (Glynn & Sandman, 2012) fetal movement response to the VAS was assessed at 25, 31, and 37 gestational weeks in 190 mother–fetus pairs. Nearly all fetuses showed a movement response to the VAS at 25 gestational weeks (unlike FHR) with evidence of increasing maturation in the response at 31 and 37 weeks. Fetuses exposed to elevated cortisol early in gestation failed to respond to the VAS at 25 gestational weeks. However, fetal exposure to elevated cortisol later in gestation, near term, was associated with a larger response. The gestational timing effects for exposure to cortisol observed in this study of fetal behavior are consistent with findings we have seen in infants and children who were exposed to maternal cortisol during specific gestational intervals and will be discussed in later sections.

There is evidence that fetal exposure to maternal pituitary peptides during the third trimester also exerts programming influences on fetal behavior. In a study of 135 maternal–fetal dyads, we (Sandman et al., 2003) reported that fetal exposure to elevated levels of maternal endogenous opioids (derived from the POMC prohormone) was associated with significantly lower rates of FHR habituation to repeated VAS. In contrast, fetuses in maternal environments with elevated ACTH (also from the POMC molecule) exhibited the most rapid rate of habituation. Enzymatic cleavage of POMC typically liberates these two peptides simultaneously; however, uncoupling does result in different patterns of exposure. Different patterns can result from several sources including stress induced levels of CRH (Laatikainen, Virtanen, Kaaja, & Salminen-Lappalainen, 1991) and 3D conformation of the POMC molecule (Rosenblatt & Dickerson, 1997). It is remarkable that the behavior of the human fetus is sensitive to these subtle variations in the maternal environment. These findings in the human fetus, in conjunction with those for CRH and cortisol, provide strong and perhaps the only support for the argument that there are programming influences on the fetal nervous system at all levels of the HPA-placental axis.

Fetal Development and Birth Phenotype

A central tenet of the fetal programming hypothesis is that birth phenotype, itself, is not the primary source of risk but instead reflects adverse *in utero* exposures that influence fetal development and contribute to poor birth outcomes. Research by DiPietro et al. (2015) highlights the continuity between fetal and postpartum life as an important reason to study the fetus. Knowledge about fetal growth and development and perhaps the trajectory of both may foreshadow subsequent postpartum development. For instance there are studies reporting continuity between patterns of fetal heart rate (FHR) and movement and infant mental and motor development (Almli, Ball, & Wheeler, 2001; DiPietro, Bornstein, Hahn, Costigan, & Achy-Brou, 2007; Groome et al., 1999; Leader, Baillie, Martin, & Vermeulen, 1982; Vlastos et al., 2007; Werner et al., 2007), infant temperament (DiPietro et al., 2002; DiPietro, Costigan, & Pressman, 2002; DiPietro, Hodgson, Costigan, & Johnson, 1996; Werner et al., 2007), and infant autonomic function (DiPietro, Costigan, Pressman, & Doussard-Roosevelt, 2000; Kisilevsky & Muir, 1991). Little is known about whether fetal measures of neurological development are associated with birth outcomes.

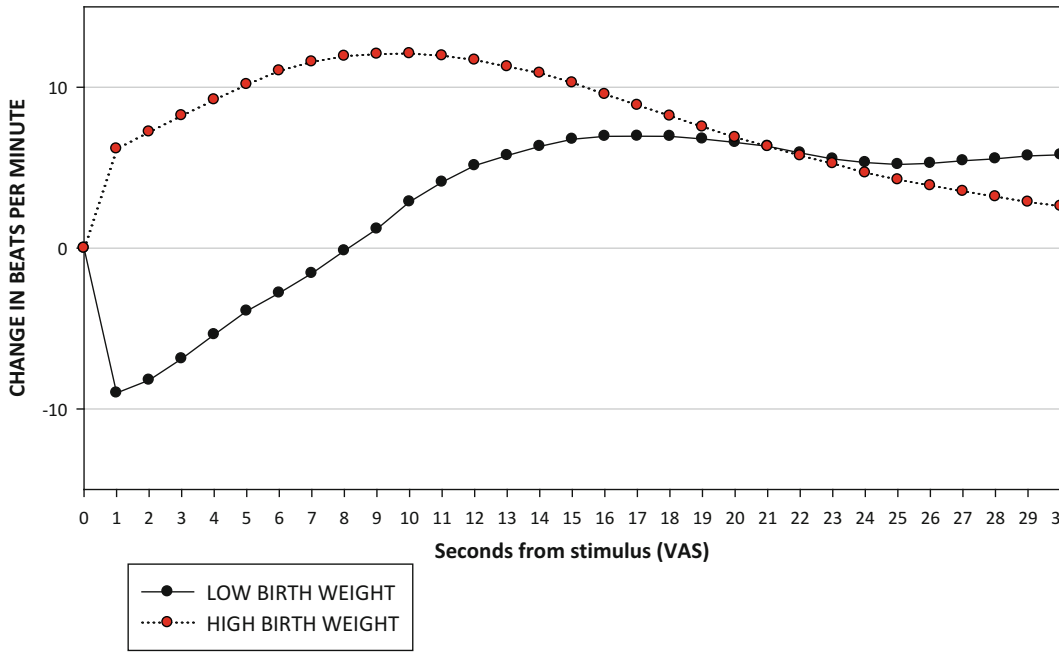

Fig. 13.4 Two significantly different patterns of change in fetal heart rate (*Y* axis) after presentation of a VAS at ~30 weeks of gestation predict eventual birth weight. The pattern of immediate deceleration followed by gradual recovery is associated with low birth weight

We (Sandman, Cordova, Davis, Glynn, & Buss, 2011) wondered if fetal responses associated with neurological maturity could be an early signal or risk indicator of poor birth outcome (DiPietro, Irizarry, Hawkins, Costigan, & Pressman, 2001; Hepper, 1995). Group based trajectory analysis of fetal heart rate in response to a VAS resulted in five distinctive profiles of response and recovery among a sample of 174 maternal–fetal dyads. Two FHR patterns at 30 weeks of gestation were significantly associated with subsequent birth weight. The group of fetuses with an immediate FHR deceleration to the VAS followed by an immediate acceleration that persisted for over 30 s had the lowest birth weight. A group characterized by immediate and fast acceleration to the VAS followed by a slow recovery to baseline was associated with the highest birth weight (see Fig. 13.4). *Fetal size* at the time of FHR assessment was not related to the heart rate response patterns. It is possible that fetal neurological maturation itself reflects or influences size at birth (2 months after these responses were recorded) or that this association reflects a systemic relation between birth phenotype and fetal neurological maturation. For instance, common factors might influence both fetal neurological maturity and birth outcomes and account for this association. However, there are well-established neurological consequences associated with low birth weight and the current findings suggest that these consequences may have their origins in early fetal development rather than as a result solely of birth outcome. Thus, this is evidence that low birth weight with the resulting neurological and health risks may be continuous with, and may be detected from, early fetal neurodevelopmental behavioral patterns.

As discussed above, the important and fertile initial studies introducing the programming hypothesis were retrospective and relied on birth phenotype, either birth weight or gestational length, as markers of unfavorable intrauterine exposures. In addition to the health risks described above, there are well-documented increases in infant and toddler mortality and

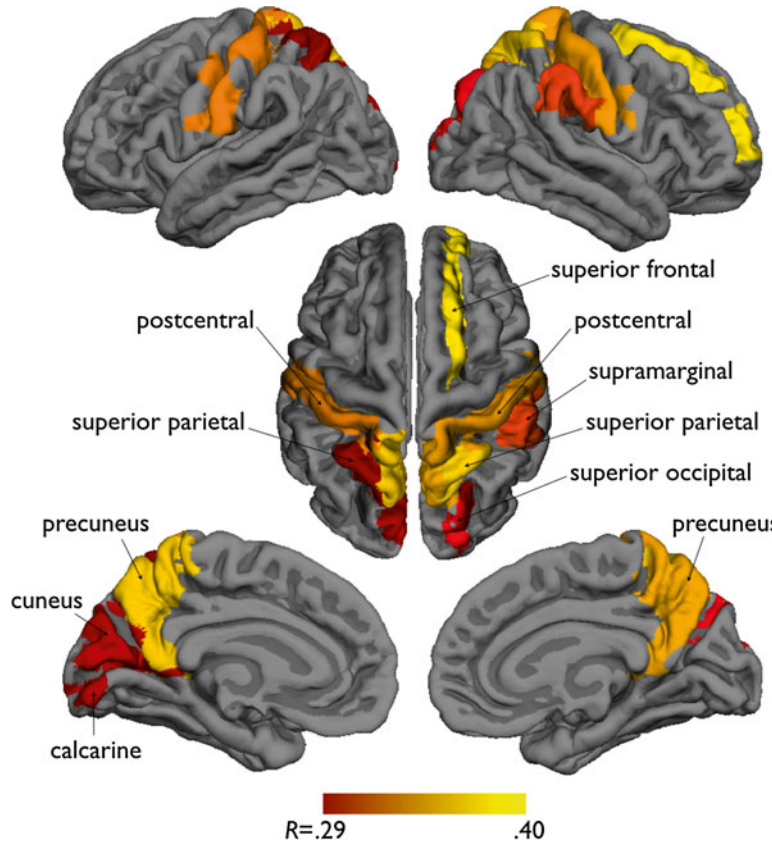

Fig. 13.5 Network efficiency was significantly associated with the length of gestation at local brain regions. Strong positive associations (*red* to *yellow*) were found in the bilateral precuneus, superior parietal, and postcentral regions including cuneus, supramarginal, calcarine, superior frontal and occipital regions ($p < 10–4$ with FDR correction; age and gender covariates). (Reprinted with permission from: Kim et al., 2014, *Neuroimage, 100C*, 619–627)

morbidity associated with preterm birth. Consequences of shortened gestation are not restricted to preterm birth, but are apparent across the full gestational age range (Spong, 2013). New findings from our program indicate that there are neurological advantages of longer gestation even in full-term births (greater than 37 weeks). Imaging studies (MRI and DTI) from our group indicated that longer gestation, up to 41 weeks, is associated with increased cortical volume (Davis et al., 2011) and neural efficiency (Kim et al., 2014) in 6- to 10-year-old children (see Fig. 13.5). These studies are consistent with the hypothesis that stress signals that influence gestational length also program the developing central nervous system. We discuss below evidence that psychobiological stress signals shape the developing CNS with consequences for neonatal, infant, and child emotional and stress regulation, cognition, and brain development.

Prenatal Maternal Psychobiological Stress and Infant Stress Regulation

Alterations to the fetal HPA axis are frequently proposed as the primary biological pathway underlying fetal programming of later health and development. Animal studies suggest that the fetal HPA axis may be particularly vulnerable to prenatal exposure to maternal stress (Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006); however, relatively little is known about the consequences of prenatal maternal stress for HPA axis functioning among humans.

Evidence from our projects indicates that fetal exposure to maternal stress hormones exerts influences on (or programs) the development of the fetal HPA axis with consequences for neonatal functioning (Davis, Glynn, Waffarn, & Sandman, 2011). In one study, maternal plasma cortisol and report of stress, anxiety, and depression were

assessed at five intervals in 116 women throughout pregnancy. Their full-term infants were assessed at 24 h after birth. Infant cortisol and behavioral responses were evaluated to the painful stress of a clinically indicated heel-stick blood draw. We found that a larger infant cortisol response to the painful procedure was associated with previous (fetal) exposure to elevated concentrations of maternal cortisol during the late second and third trimesters. Further, fetal exposure to elevated maternal cortisol early in pregnancy and elevated maternal psychological distress throughout pregnancy were associated with a slower rate of behavioral recovery from the pain of the heel-stick. The results of this study indicate that the trajectory of maternal cortisol levels across gestation predicts infant behavioral and physiological responses to stress.

In further support of the hypothesis that maternal cortisol is an underlying mechanism related to programming of the fetal HPA axis, we have shown that prenatal administration of the synthetic glucocorticoid betamethasone is associated with a similar pattern of infant HPA axis reactivity (Davis, Waffarn, & Sandman, 2011). In a separate cohort, 90 full-term infants were recruited into two groups; one group was treated with betamethasone, and the other was a matched comparison group. Consistent with the findings reported above, infants exposed to prenatal glucocorticoids displayed a larger cortisol response to the heel-stick procedure. Moreover, infants exposed to synthetic glucocorticoids earlier in gestation (range was 24–34 weeks GA) displayed the largest cortisol response to the painful heel-stick procedure.

These complementary studies are important because they indicate that fetal exposure to maternal glucocorticoids and to some extent psychological stress alters neonatal stress responses. Moreover, the findings for glucocorticoids provide support that the timing of fetal exposures during gestation determine the neonate's ability to respond behaviorally and physiologically to stressors in the postnatal environment. The large and prolonged cortisol response and protracted behavioral responses to the stress of painful stimulation reported in these two studies may be indicative of a dysfunctional stress system. This is particularly likely given the cost associated with an inability to turn off the stress response after the stressor has passed (McEwen, 1998). Growing evidence indicates that prenatal influences play a role in the development of psychiatric disorders such as anxiety, depression, and externalizing behavior problems (Bohnert & Breslau, 2008; Costello, Worthman, Erkanli, & Angold, 2007; Hellemans, Sliwowska, Verma, & Weinberg, 2010). It has been proposed that disruptions to HPA axis functioning may be responsible for this effect (Kapoor, Petropoulos, & Matthews, 2008; Seckl & Meaney, 2006). The two studies described here demonstrate that prenatal glucocorticoids alter the functioning of stress regulatory systems in the offspring, independent of postpartum influences, and may be a potential mechanism for fetal programming of later psychiatric disorders. Longitudinal follow-up of these infants, as described below, provides further evidence for the role of the fetal environment in shaping trajectories associated with later psychological well-being.

Prenatal Maternal Stress and Infant Emotional and Cognitive Development

Research from our laboratory as well as from several international laboratories provides support for the influence of fetal exposures on developmental trajectories. Building on the findings discussed above, one of the most consistent findings is that fetal exposure to psychological and biological stress signals is associated with more reactive physiological and behavioral responses to challenge perhaps reflecting more fearful temperament during infancy (Bergman, Sarkar, O'Connor, Modi, & Glover, 2007; Buss et al., 2012; Davis et al., 2005, 2007; Davis, Buss et al., 2011; Davis & Sandman, 2012; Davis, Sandman, Buss, Wing, & Head, 2013; de Weerth, Van Hees, & Buitelaar, 2003; O'Connor, Bergman, Sarkar, & Glover, 2013; O'Connor, Heron, Golding, & Glover, 2003; Van den Bergh, 1990; Van den Bergh & Marcoen, 2004; Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008). Studies with the neonate indicate that fetal exposures influence neurodevelopment and we have

observed that these exposures are related to cognitive development during infancy. As we report in detail below, fetal exposure to elevated maternal cortisol and pCRH as well as maternal reports of psychological distress independently predicted infants' cognitive development and temperament.

In one of our earliest studies of fetal programming effects on infant outcome, the effects of maternal antenatal and postnatal anxiety and depression on direct laboratory measures of infant negative behavioral reactivity were examined in a sample of 22 mother–infant pairs (Davis et al., 2004). Maternal state anxiety and depression were assessed during the third trimester of pregnancy and 4 months postpartum. We reported that elevated maternal general anxiety and depression during the prenatal, but not the postnatal period, were related to increased infant negative behavioral reactivity to novelty. We replicated these findings in a larger sample of 247 mothers and their full-term infants in which we measured perceived stress, depressive symptoms and general anxiety at 20, 25 and 30 gestational weeks and maternal report of infant negative reactivity at 2 months of age (Davis et al., 2007). Elevated maternal reports of prenatal anxiety and depressive symptoms at each gestational interval predicted increased infant negative reactivity. However, only the significant associations between maternal depressive symptoms remained after controlling for postnatal maternal psychological state.

These encouraging findings have been followed up in subsequent studies with older infants and with pregnancy specific anxiety included in addition to measures of general anxiety. In a sample of 120 healthy, 2-year-old children, maternal anxiety was measured five times during pregnancy and infant temperament was measured at 2 years (Blair, Glynn, Sandman, & Davis, 2011). Higher reported maternal pregnancy specific anxiety between 13 and 17 weeks of gestation (using growth curve analysis) was associated with increased negative emotionality in the infants. This association remained significant after adjusting for postnatal maternal anxiety, demographic, or obstetric factors. These findings indicated that fetal exposure to maternal pregnancy specific anxiety early in gestation may be a sensitive period for programming effects on infant temperament and are consistent with a theme emerging in our research that pregnancy specific anxiety is more directly related to child outcomes compared to more general measures of stress and anxiety.[2]

Together, these studies illustrate that fetal exposure to maternal psychological distress, and perhaps specifically maternal depressive symptoms and pregnancy specific anxiety are linked to increased behavioral reactivity during infancy. Interestingly, as discussed above, psychological and biological distress measures independently predicted infant outcomes. In the sample described above of 247 mothers and their full-term infants, we found that elevated maternal cortisol at 30 gestational weeks (Davis et al., 2007) and elevated concentrations of pCRH at 25 gestational weeks (Davis et al., 2005) was significantly associated with greater maternal report of infant negative reactivity. Importantly these findings are independent of birth outcome, sociodemographic factors and postnatal maternal psychological measures. These novel findings highlight the importance of considering both maternal (cortisol) and fetal pCRH signals. These data suggest that fetal exposure to stress signals is associated with behavioral reactivity during infancy.

In contrast to the highly consistent set of findings linking prenatal stress to emotional and stress reactivity, there is less consensus in the literature regarding the association with cognitive outcomes. There is evidence that maternal self report of elevated stress and anxiety as well as exposure to traumatic life events, such as severe ice storms, during pregnancy are associated with delayed infant and child cognitive, language, and neuromotor development (Cao, Laplante, Brunet, Ciampi, & King, 2014; Laplante et al., 2004; Laplante, Brunet, Schmitz, Ciampi, & King, 2008; Walder et al., 2014). However, there is a report that modest elevations in psychosocial stress during late gestation may enhance cognitive maturation

[2] It is unknown why measures of distress relevant to pregnancy have a greater impact on child outcome than measures of generalized anxiety. But it is probable that a woman's fears, beliefs and concerns about pregnancy has direct and local implications for well-being and is the primary source of anxiety during pregnancy. As such, it may be the most important psychosocial index of anxiety that can affect birth and child outcome.

(DiPietro, Novak, Costigan, Atela, & Ruesing, 2006). It is plausible that some of these inconsistencies are related to a focus on general distress rather than pregnancy specific anxiety and the differential influence of timing and trajectories of stress exposures over gestation.

In one study, we examined the consequences of fetal exposure to biological and psychological maternal stress for cognitive and motor development (Bayley Scales of Infant Development) in 125, healthy full-term infants at 3, 6, and 12 months of age. Trajectories of maternal cortisol and psychological state were constructed from measures taken five times during pregnancy. Fetal exposure to elevated concentrations of cortisol and higher maternal reports of pregnancy specific anxiety early in gestation (13–15 weeks GA) were associated with a slower rate (slope) of mental development over the 1st year, and lower mental development scores at 12 months (there were no effects for motor development). Elevated levels of maternal cortisol later in gestation (>37 weeks GA), however, were associated with accelerated cognitive development and higher scores at 12 months. Together, levels of maternal cortisol early and late in gestation accounted for 8 % of the variance in infant cognitive performance. At 13 weeks GA, a 0.1 μg/dl *increase* in cortisol was associated with a 4-point *decrease* in MDI. At >37 weeks GA, a 0.1 μg/dl *increase* in cortisol was associated with a 2-point *increase* in MDI. Despite the finding that fetal exposure early in pregnancy to both maternal cortisol and pregnancy-specific anxiety had similar effects on infant mental development, these two measures were not correlated with each other and they exerted independent influences on development. As discussed above, this finding is consistent with the majority of published studies that document independent contributions of concurrent assessments of maternal psychological state and maternal cortisol during pregnancy (Davis et al., 2007; de Weerth & Buitelaar, 2005; McCool, Dorn, & Susman, 1994; Petraglia et al., 2001).

Our data evaluating fetal programming of infant development highlight several consistent themes. First, psychological and biological stress measures independently predict infant development. Second, among the psychological measures, maternal depression and maternal pregnancy specific anxiety most strongly predict infant outcomes. Third, these findings are consistent with evidence from the fetus and neonate that maternal stress signals "program" increased reactivity perhaps leading to more fearful temperament in the offspring. It is plausible that there will be long term consequences associated with these infant outcomes. Infants who are easily aroused by varied stimulation are more likely to become behaviorally inhibited as young children (Kagan, Snidman, & Arcus, 1998; Pfeifer, Goldsmith, Davidson, & Rickman, 2002) and result in higher risk for social anxiety in adolescence (Schwartz, Snidman, & Kagan, 1999). Further, maternal stress signals are associated with cognitive development during infancy which has consequences for scholastic and later life success. We have begun to follow these infants into childhood to examine the persisting influences of fetal programming on affective, cognitive, and neurological integrity.

Prenatal Stress, Child Development and Neurological Mechanisms

Prenatal Programming of Emotional and Behavioral Problems

Our continued assessment of these cohorts during preadolescence provides consistent evidence that one of the primary consequences of fetal exposure to maternal distress during pregnancy is increased fearful and anxious behavior in infants and early childhood. In our cohort of 178 mother–child pairs, we (Davis & Sandman, 2012) investigated the consequences of prenatal exposure at 19, 25, and 31 gestational weeks both to maternal biological stress signals and psychological distress on anxiety in preadolescent children. Anxiety was evaluated in the children at 6–9 years of age using the Child Behavior Check List (CBCL). Outcome was adjusted by considering obstetric risk, sociodemographic factors, and postnatal maternal psychological distress. We found that prenatal exposure to elevated maternal reports of depression, perceived stress, and pregnancy-specific anxiety (but not general anxiety) were associated with increased anxiety in children. However, when we

evaluated child anxiety status using clinical cutoffs, logistic regression revealed that among all of the psychological stress measures, only pregnancy-specific anxiety was associated with increased risk of child anxiety within a borderline/clinically significant range. A one-point change in pregnancy anxiety (range 10–40) increased childhood risk for exhibiting anxiety by 10 %. Elevated maternal cortisol across gestation also was associated with increased risk of anxiety among the children and this risk was independent from the effects of pregnancy specific anxiety and other postnatal psychosocial confounding factors. Children with anxiety ratings within the borderline/clinically significant range were twice as likely to have been exposed to higher maternal cortisol during gestation compared to children with ratings in the normal range (odds ratio = 2.1, 95 % confidence interval = 1.1—3.9, $p < 0.05$). Interestingly, the association between prenatal cortisol and child anxiety was observed primarily among females (Sandman, Glynn, & Davis, 2013).

Exposure to maternal glucocorticoids during gestation may influence the development of anxiety by modifying fetal development in regions such as the amygdala (Herman & Cullinan, 1997; Joels & Baram, 2009) that are particularly sensitive to excessive levels of glucocorticoids (Rodrigues, LeDoux, & Sapolsky, 2009) and play a role in the regulation of anxious behavior (Schulkin, 2006). Findings from animal models illustrate that prenatal stress exposures including excess glucocorticoids alter the density of cortisol receptors (Kapoor et al., 2006) and increase the production of CRH in the amygdala (Cratty, Ward, Johnson, Azzaro, & Birkle, 1995; Mueller & Bale, 2008). Further, exposure to stress during the prenatal period is associated with an increase in amygdala volume (Salm et al., 2004), suggesting a plausible mechanism by which prenatal cortisol may influence vulnerability to the development of anxiety. We evaluated the consequences of fetal exposure to maternal cortisol early in pregnancy on the volumes of the amygdala and hippocampus in 65 children ages 6–9 years and on child affective problems (Buss et al., 2012). We found that higher maternal cortisol concentrations in early gestation were associated with a larger right amygdala volume in girls but not boys (Fig. 13.6). Moreover, higher maternal cortisol levels in early gestation were associated with more affective problems in girls, and this association was medi-

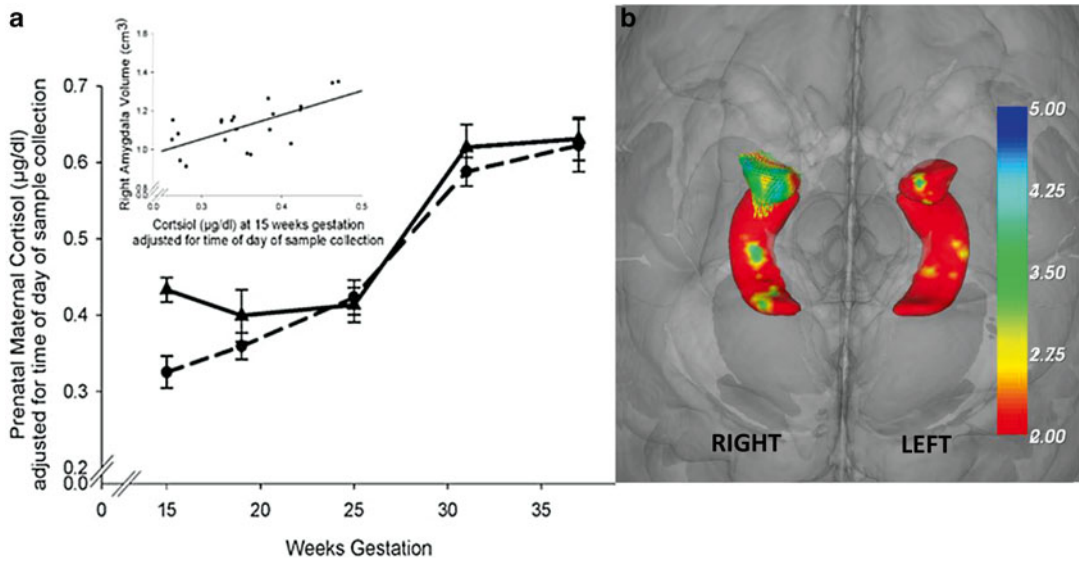

Fig. 13.6 (**a**) Relations between fetal exposure to maternal cortisol across gestation and volume of the right Amygdala of girls at 6–8 years of age. (*Filled triangle*, subjects with large amygdala; *filled circle*, subjects with small amygdala.) Significant differences in cortisol are at 15 weeks of gestation. *Insert* illustrates relation between right amygdala volume and cortisol levels at 15 weeks of gestation. (**b**) Stylized dorsal view of manually traced hippocampus and amygdala illustrating the significance (*cold colors*) only in the volume of the right amygdala of girls

ated by amygdala volume. The magnitude of the effect was substantial; a 1 standard deviation increase in maternal cortisol was associated with a 6.4 % increase in the size of the right amygdala. The effect was significant after adjusting for the effects of other pregnancy, birth, and child and maternal characteristics. There was no association between hippocampal volume and prenatal exposure to cortisol. Statistical modeling further suggested that the association between maternal cortisol in early gestation and affective problems in girls is mediated by their cortisol-associated larger right amygdala.

These findings may be the first (and only) report linking maternal stress hormone levels in human pregnancy with subsequent child amygdala volume and emotional development and may provide an important clue to the origins of neuropsychiatric disorders. For instance our findings in healthy children approaches the differences in amygdala volume between clinically depressed patients and healthy comparison volunteers (Lange & Irle, 2004) and are consistent with findings that exposure to high levels of stress in early postnatal life has been associated with altered development and function of the amygdala (Lupien et al., 2011; Mehta et al., 2009; Tottenham et al., 2010).

To further explore the possibility that glucocorticoids are a mechanism that may be associated with risk for affective problems, we recruited a sample of children who were born full term and with a known fetal exposure to synthetic glucocorticoid treatment. Children who were exposed as fetuses to glucocorticoid treatment had significantly thinner cortices, primarily in the rostral anterior cingulate (rACC), a region that plays a critical role in stress and emotional regulation (Blair et al., 2012; Etkin & Schatzberg, 2011). Not only was more than 30 % of the rACC thinner among children with fetal exposure to glucocorticoid treatment, but the magnitude of the effect was substantial. The rACC was 8–9 % thinner among glucocorticoid-treated children (see Fig. 13.7). The possible clinical significance of this association is underscored by the observation that there is a 10–14 % reduction in the rACC among children with depressive symptomatology (Boes, McCormick, Coryell, & Nopoulos, 2008). Consistent with the possibility that these neurologic changes indicate prodromal risk for mental health problems, we reported that a thinner rACC in these children was a significant mediator of increased child affective problems. Notably, the significant associations were observed among children who are healthy and born at term, and these findings could not be explained by sociodemographic (income or education) or maternal (depression, IQ) factors

Although much of the literature has focused on the role of fetal exposure to psychobiological stress on internalizing problems, there is recent evidence that maternal depression during the prenatal period is associated with risk for externalizing behavior problems. For example, Barker, Jaffee, Uher, and Maughan (2011) reported that prenatal maternal depression but not anxiety or postpartum depression, significantly increased the risk for externalizing behaviors and was associated with significantly lower verbal intelligence in a large sample ($N=3298$). In another large study ($N=5029$), prenatal but not postnatal depressive symptoms were associated with lower child intelligence (Evans et al., 2011). Early exposures to maternal depression, including prenatally, were independently associated with externalizing behaviors and low social competence in adolescent boys (Korhonen, Luoma, Salmelin, & Tamminen, 2012).

Maternal depression is one of the most common prenatal complications and the consequences of fetal exposure to maternal depression are poorly understood. In a group of 81 children, ages 6–9 years, who had been followed beginning at 19 gestational weeks, we assessed the association between fetal exposure to maternal depression and cortical thickness (using FreeSurfer) and the implications for child behavioral problems (Sandman, Buss, Head, & Davis, 2015). Significant cortical thinning in children primarily in the right frontal lobes was associated with exposure to prenatal maternal depression at each gestational interval. The strongest association, however, was observed in children who were exposed to maternal depression at 25 gestational weeks. Fetal exposure to maternal depression at 25 gestational weeks was associated with cortical

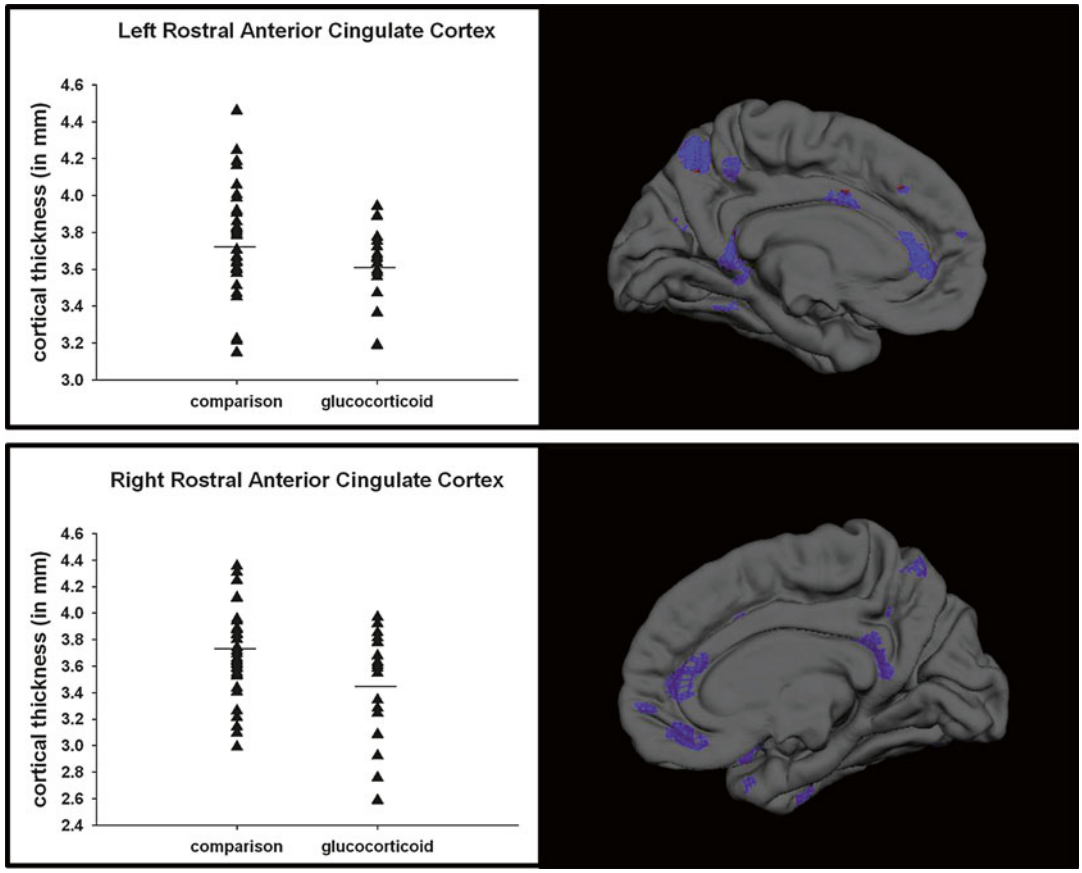

Fig. 13.7 Fetal exposure to synthetic glucocorticoids is significantly associated with cortical thinning among preadolescent children who were full term at birth. *Blue* overlays represent areas of significant cortical thinning after FDR correction for multiple comparisons. The strongest association was in the rACC, which was 8–9 % thinner among glucocorticoid-treated children compared to the comparison group (Adapted from Davis et al. 2013)

thinning in 19 % of the whole cortex and 24 % of the frontal lobes, primarily in the right superior, medial orbital and frontal pole regions of the prefrontal cortex (Fig. 13.8). In this cohort, exposure to prenatal maternal depression and cortical thinning both were associated with child externalizing behavior. A formal test of mediation and a focus on the prefrontal cortex indicated that the relation between prenatal maternal depression and child externalizing behavior was mediated by thinning in the frontal areas.

The pattern of cortical thinning in children exposed to prenatal maternal depression is similar to patterns in depressed patients (Fallucca et al., 2011; Peterson & Weissman, 2011; Tu et al., 2012) and in individuals with risk for depression (Peterson et al., 2009). Vulnerability at 25 gestational weeks to prenatal depression has been reported previously (Sandman & Davis, 2012; Yim et al., 2009) and may result from the enormous growth and dramatic structural changes in the nervous system during this interval. Further, this gestational period is a sensitive window for the neurodevelopmental effects of pCRH (Davis et al., 2005; Yim et al., 2009).

The mechanism by which maternal depression is transmitted to the fetus is unknown. Maternal cortisol does not appear to be the signal for the effects of maternal depression on fetal neurological development (Fetal exposure to maternal

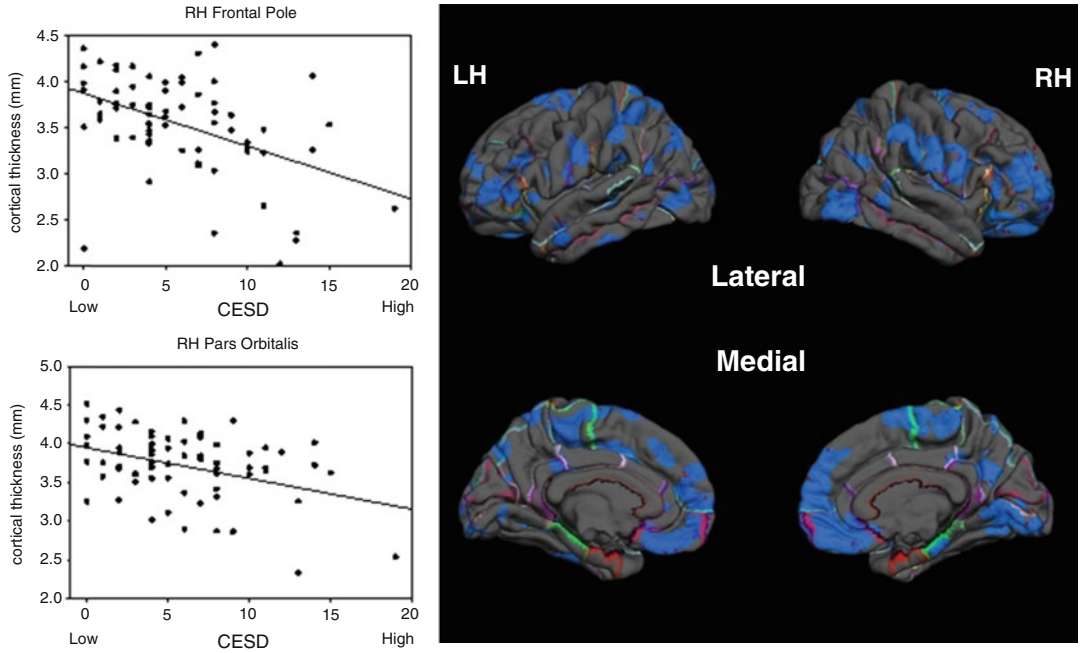

Fig. 13.8 Lateral and medial pial maps that illustrate that fetal exposure to maternal depression at 25 weeks of gestation is associated with cortical thickness in 6- to 8-year-old children. The *cooler colors* in the FDR corrected maps illustrate that fetal exposure to maternal depressive symptoms are associated with widespread cortical thinning in children (19 % of the whole cortex and 24 % of the frontal lobes) (*LH* left hemisphere, *RH* right hemisphere). Two representative scatter plots depict the association between maternal depressive symptoms at 25 weeks of gestation and cortical thickness in children at 6–8 years of age [*RH* frontal pole, $r71 = -0.46$, $p < 0.001$; RH Pars Orbitalis, $r71 = -0.39$, $p < 0.001$] (Adapted from Fetal exposure to maternal depressive symptoms is associated with cortical thickness in late childhood Biological Psychiatry, 2015 doi:10.1016/j.biopsych.06.025, 2014)

depressive symptoms is associated with cortical thickness in late childhood, Sandman et al, 2015). Future studies to determine plausible biological signals of psychological stress are a high priority.

Prenatal Programing of Cognitive Development

As discussed previously, less is known about the implications of gestational stress for child cognitive development. Research by Van den Bergh et al. (2008) provides evidence for consequences of fetal exposures to anxiety on executive function that persist into adolescence. We have had the unique opportunity to assess the influence of fetal exposures to maternal stress in our cohort as they have moved into preadolescence. We assessed working (sequential) memory and inhibitory control (flanker task) as representing core elements of executive function in 89 children (ages 6–9 years) followed from 15 gestational weeks (Buss, Davis, Hobel, & Sandman, 2011). Children exposed as fetuses to high levels of maternal pregnancy specific anxiety had lower scores on both measures of executive function. Exposure to pregnancy specific anxiety in early pregnancy explained 10 % of the variance on simple (congruent) trials and 17 % of the variance on the incongruent trials of the flanker task (Fig. 13.9). Although fetal exposure to higher state anxiety and depression also were associated with lower visuospatial working memory performance, neither explained additional variance after accounting for pregnancy-specific anxiety.

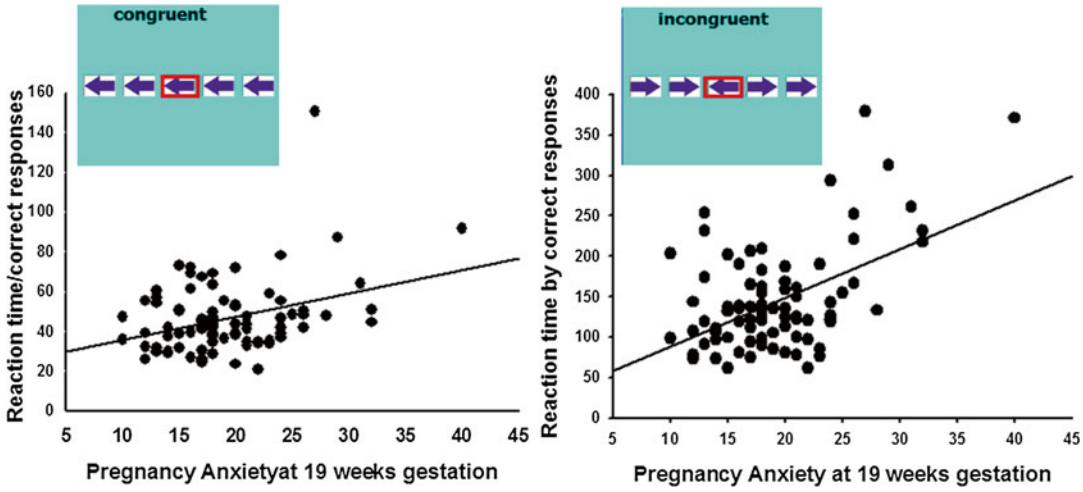

Fig. 13.9 Association between fetal exposure to pregnancy-specific anxiety (PSA) at 19 weeks of gestation and a measure of executive function (speed/accuracy trade-off) in 6- to 8-year-old children. Exposure to pregnancy specific anxiety explained 10 % of the variance on congruent trials (response to the target [*red box*] was identical to the flankers) and 17 % of the variance on the incongruent trials (correct response to the target was opposite to the flankers)

These findings are consistent with our findings in a second, separate cohort showing that pregnancy specific anxiety early in pregnancy is associated with cognitive development at 1 year of age and provide strong support for a persisting association between fetal exposure to mother's anxiety about the course of pregnancy and cognitive function in preadolescent children.

We have reviewed evidence in this chapter that pregnancy-specific anxiety exerts programming influences on birth outcome, infant and child emotional regulation, and infant and child cognitive development. One direction for future research is the evaluation of the mechanisms by which pregnancy-specific anxiety (PSA) influences neurodevelopment. As discussed, we recently have shown that pregnancy-specific anxiety is related to maternal cortisol trajectories over gestation. In a relatively small sample we evaluated fetal exposure to pregnancy-specific anxiety on brain morphology in 6- to 9-year-old children. Structural MRI scans were conducted in 35 children who had been followed with measures of PSA beginning at 19 weeks GA (Buss, Davis, Muftuler, Head, & Sandman, 2010). With the application of voxel-based morphometry, we found pregnancy anxiety at 19 weeks of gestation was associated with gray matter volume reductions in the prefrontal cortex, the premotor cortex, the medial temporal lobe, the lateral temporal cortex, the postcentral gyrus as well as the cerebellum extending to the middle occipital gyrus and the fusiform gyrus. These effects were significant after adjusting for total gray matter volume, age, gestational age at birth, handedness, and postpartum maternal stress. This prospective study is among the first and perhaps only study to show that a temporal pattern of fetal exposure to pregnancy anxiety was related to specific changes in brain morphology. The timing of the pregnancy-specific anxiety's influence on brain structure was consistent with several other outcomes related to fetal exposure to pregnancy-specific anxiety reviewed above. The diffuse alteration of brain systems in children exposed to prenatal stress may moderate the association between maternal distress and behavior that has been reported.

Challenges and Future Directions

Programming and Genetic Inheritance

A lingering issue for the programming or developmental origins of health and disease models is the role of genetic inheritance. For instance, for a mother who is depressed during pregnancy and has a child who exhibits unique traits and patterns of brain development, it is possible that (1) the effects on the offspring are related to the stress on the fetus of mother's emotional condition or (2) that a common genetic factor is related both to maternal prenatal depression and to child outcome. This is a difficult confound to unwind. There are several lines of evidence from research with humans that suggest that shared genes alone cannot account for fetal programming effects. First, genetically informed study designs involving children conceived by in vitro fertilization who were not genetically related to their mothers provide strong evidence that the early environment contributes to child mental health (Lewis, Rice, Harold, Collishaw, & Thapar, 2011; Rice et al., 2010). Second, studies evaluating the consequences of random exposure to extreme stressors during gestation suggest that prenatal exposures to stress exerts a lasting influence on birth outcome and child development (Huizink et al., 2007; King & Laplante, 2005; Lewis et al., 2011; Li et al., 2010; Rice et al., 2010). Third, fetal exposure to synthetic glucocorticoid administration exerts similar consequences to elevated endogenous maternal cortisol (Davis et al., 2013; Davis, Buss et al., 2011). These findings in conjunction with experimental animal research provide strong evidence for fetal programming effects beyond shared genes.

Even stronger support for a programming influence from mother to human fetus comes from the study of monozygotic (MZ) twins. MZ twins may share (monochorionic—MC) or have their own placenta (dichorionic—DC). Approximately 30–35 % of the MZ twins are DC. MC-MZ twins almost always share between them large artery-to-artery and/or vein-to-vein vascular anastomoses (allowing the exchange of blood between both members of the pair; Benirschke & Kaufmann, 1995). There are greater intrapair differences between healthy DC-MZ twins than between healthy MC-MZ twins. For instance, in 7-year-old Caucasian twins, the intrapair difference of IQ was greater in MZ twins with separate placentas than in MZ twins who shared a placenta (Melnick, Myrianthopoulos, & Christian, 1978.) Greater intrapair differences for DC-MZ compared to MC-DZ twins also were reported for measures of arithmetic and vocabulary in 8- to 14-year-old children (Jacobs et al., 2001). In 4- to 6-year-old twins, intrapair differences were significantly different in DC-MZ twins on many measures of cognitive ability and personality (Sokol et al., 1995). Within-pair variability and mirroring of fingerprints was reported to be greater among DC-MZ compared to MC-MZ twins (Davis, Phelps, & Bracha, 1995). What is so important for the programming model about this collection of findings is that these differences in child outcome, arguably related to different sources of fetal information (via the placenta) is that they cannot be due to genetic differences, because all pairs are monozygotes, nor can they be due to parity, gestational age, birth weight, or maternal education—and other important covariates. The relative discordance in DC twins is because they are sampling the maternal milieu with their own placenta.

Alternative Models to Fetal Programming

There are alternatives to the fetal programming and developmental origins of disease models as presented and supported in this chapter. For instance, the stress-inoculation model predicts that exposure to mild adversity during early development promotes resilience in the face of stressful (i.e., congruent) circumstances later in life (Lyons & Parker, 2007). The adaptive calibration model (Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van Ijzendoorn, 2011) argues that early exposure to adversity increases the lability of responses to subsequent adversity. This results in increased impairment after exposure to stress later in life but also enhanced ability to benefit from supportive and protective features of the environment. The Predictive Adaptive Response (PAR) Model

(Gluckman & Hanson, 2004a, 2004b; Gluckman, Hanson, & Spencer, 2005) predicts that optimal outcomes are related to the congruence between the prenatal and postnatal environments. A discordant transition between fetal and infant life places the child at risk for health complications. For instance, infants provided with sufficient nutrition after near-starvation in utero have an increased risk of developing metabolic diseases (Armitage, Taylor, & Poston, 2005; Cleal et al., 2007).

We (Sandman, Davis, & Glynn, 2012b) tested the consequences of prenatal and postnatal maternal reports of depressed mood on infant cognitive development. In addition to the developmental effects of fetal exposure to prenatal maternal depression reviewed above, infants suffer pervasive negative consequences when exposed to postnatal maternal depression (Fihrer, McMahon, & Taylor, 2009; Kurstjens & Wolke, 2001), even if their mother's depressive symptoms are subclinical (Moehler et al., 2007). To test the PAR model, we assessed symptoms of maternal depression at regular intervals throughout the pregnancies of a sample of 221 healthy pregnant women. After delivery, mothers and infants were evaluated with the Bayley Scales of Infant Development at 3, 6, and 12 months. Infants were separated into four groups. Two groups included infants whose mothers had concordant prenatal and early postnatal depressive symptoms: either high prenatal and postnatal symptoms (concordant adversity) or low prenatal and postnatal symptoms (concordant favorability). The other two groups included infants whose mothers had discrepant prenatal and postnatal depressive symptoms: either high levels of prenatal symptoms and low levels of postnatal symptoms (prenatal-only adversity) or low levels of prenatal symptoms and high levels of postnatal symptoms (postnatal only adversity). We found that infants thrive, at least on critical dimensions of psychomotor and mental development, when their prenatal and postnatal environments are congruent, even if the conditions of those environments are adverse. Specifically we found increased motor and mental development during the first year of life among infants whose mothers experienced congruent levels of depressive symptoms during and after pregnancy, even when the levels of symptoms were relatively high and the prenatal and postnatal environments were unfavorable. These results for maternal mood support the PAR model and are consistent both with reports that exposure to incongruent prenatal and postnatal nutritional conditions increases infants' risk of developing metabolic disease and with findings of adaptive advantages among infants exposed to matching prenatal and postnatal nutritional environments. We concluded that under some circumstances, congruence between prenatal and postnatal environments, rather than the main effect of adversity, prepares the fetus for postnatal life and confers an adaptive advantage for critical survival functions during early development.

Sex Differences in Fetal Programming

Historical records and current studies (Cooperstock & Campbell, 1996; Mathews & Hamilton, 2005), consistently confirm that more males than females are conceived (primary sex ratio) and born (secondary sex ratio). This suggests that under certain conditions secondary sex ratio is associated with, and perhaps programmed by, preconceptional and maternal/fetal exposures to environmental events. Moreover studies consistently show that more males than females are born preterm (Cooperstock & Campbell, 1996) and that males have poorer neonatal and infant health outcomes (Peacock, Marston, Marlow, Calvert, & Greenough, 2012), have higher risk for motor and cognitive outcomes, and are less likely to survive in intensive care. It has been argued that under conditions of diminished resources fewer males are born because compared to females more resources are required to support their larger size. It is believed that the differential mortality, "programming" of morbidity and developmental impairments in males occurs early in gestation (or even preconceptionally) so that a new reproductive cycle can be initiated and that parental resources can be directed to another pregnancy (Wells, 2000). For instance, sex differences have been observed in mammalian animal models as early as meiosis. When faced with adversity, male meiosis is interrupted resulting in infertility (Hunt & Hassold, 2002). However in females, a

similar adversity does not interrupt meiosis resulting in greater chances of survival but with the possible risk of chromosomal abnormalities (Hunt & Hassold, 2002). There is further evidence that within weeks of implantation the female placenta is more responsive than the male placenta to changes in stress signals including detection and response to maternal glucocorticoid concentration (Clifton, 2010).

In the studies reviewed in this chapter we did not focus on if or how fetal programming findings were influenced by sex of the offspring. However, in a recent review (Sandman et al., 2013) we addressed this issue by reviewing and reanalyzing data from previously published findings (see Table 13.2). We reasoned that if biological sex itself was influenced by early environmental exposures, then the influence of

Table 13.2 Summary of studies examining sex differences in fetal programming: reanalyzed and reviewed

Study		Participants	Primary sex-specific findings
Fetus	Glynn, L. M. & Sandman, C. A. (2012). Sex moderates associations between prenatal glucocorticoid exposure and human fetal neurological development. *Developmental Science, 15,* 601–610	190 Mothers (83 female/107 male fetuses)	Relations between maternal cortisol and fetal response to vibroacoustic stimulation were stronger for females Among females, maternal cortisol was predictive of fetal behavior in response to stimulation at an earlier age than among males
Neonate	Ellman, L. M., Dunkel-Schetter, C., Hobel, C. J., Chicz-Demet, A., Glynn, L. M. & Sandman, C. A. (2008). Timing of fetal exposure to stress hormones: Effects on newborn physical and neuromuscular maturation. *Developmental Psychobiology, 50,* 232–241	158 Mothers (80 female/78 male neonates)	The association between fetal exposure to elevated maternal cortisol and placental CRH and delayed neuromuscular development was observed only among male neonates
Infant	Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research, 75,* 327–335	165 Mothers (60 female/65 male infants)	The association between exposures to elevated maternal cortisol early in gestation and impaired cognitive performance at 1-year of age was stronger among males
	Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research, 75,* 327–335	221 Mothers (103 female/118 male infants)	Congruence between exposure to maternal depression in the pre and postnatal environments was associated with advanced maturation of motor and mental abilities in 1-year-old infants. Effects were observed at an earlier age among female infants
	Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research, 75,* 327–335	248 Mothers (116 females/ 132 males)	Elevated placental CRH at 25 gestational weeks is associated with more fearful temperament and higher levels of distress behavior among female infants, but not male infants at 2 months of age
	Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research, 75,* 327–335	248 Mothers (116 females/132 males)	Increased maternal depressive symptomatology at 25 gestational weeks predicted more fearful temperament during infancy among girls, but not boys
	Grey, K. R., Davis, E. P., Sandman, C. A., & Glynn, L. M. (2012). Human milk cortisol is associated with infant temperament. *Psychoneuroendocrinology, 37,* 1224–1233	52 Mothers (27 female/25 male infants)	The positive association between milk cortisol and fearful infant temperament at 3 months of age was observed only among female infants

(continued)

Table 13.2 (continued)

Study		Participants	Primary sex-specific findings
Child	Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research, 75,* 327–335	100 Mothers (49 girls/51 boys)	The association between longer gestation and increased gray matter density is stronger among girls
	Buss C., Davis, E. P., Hobel, C. J. & Sandman, C. A. (2011). Maternal pregnancy anxiety is associated with child executive function at 6–9 years age. *Stress, 14,* 665–676	89 Mothers (39 girls/50 boys)	Pregnancy-specific anxiety predicted conflict processing in girls, but not boys
	Sandman, C. A., Davis, E. P., Buss, C., & Glynn, L. M. (2011). Exposure to prenatal psychobiological stress exerts programming influences on the mother and her fetus. *Neuroendocrinology, 95,* 7–21	35 Mothers (17 girls/18 boys)	Elevated pregnancy specific anxiety early in pregnancy is associated with reduced gray matter volumes. This effect is seen primarily in girls
	Sandman, C. A., Glynn, L. M., & Davis, E. P. (2013). Is there a viability-vulnerability tradeoff? Sex differences in fetal programming. *Journal of Psychosomatic Research, 75,* 327–335	178 Mothers (98 girls/80 boys)	The relation between prenatal maternal cortisol and child anxiety was stronger among girls
	Buss, C., Davis, E. P., Shahbaba, B., Pruessner, J. C., Head, K. & Sandman, C. A. (2012). Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *Proceedings of the National Academy of Sciences, 109*, E1312–E1319	65 Mothers (35 girls/30 boys)	Reduction in brain volumes in 6- to 9-year-old children exposed to elevated maternal cortisol early in gestation primarily was observed in girls

Adapted from Sandman et al. (2013)

environmental exposures on males and females would be programmed differently. In the majority of our studies, with a range of fetal, infant and child outcomes, we found that sex was a significant moderator. Our findings challenged the tacit assumption that females, with their adaptive flexibility early in gestation, escaped the consequences of early life exposure to adversity. Females adjust to early adversity with a variety of strategies, but their escape from the risk of early mortality and morbidity has a price of increased vulnerability for affective problems expressed later in development. The consequences of male fetal exposure to adversity threaten their viability, effectively culling the weak and the frail and creating a surviving cohort of the fittest.

Genomic Imprinting

The intimate relationship and flow of information between the fetus and mother is largely filtered by the placenta. It is not unreasonable to view the placenta as the organ of programming so it is important to understand some of the factors that influence this critical gate keeper. We have discussed several factors in this chapter but there are many others that are beyond the scope of this chapter and there are others that we are just beginning to appreciate, including genomic imprinting. Typically we inherit a working copy of a gene from each parent. For imprinted genes, one copy (either from mother or father depending on the gene) is epigenetically silenced or inactivated through the addition of methyl groups

during egg or sperm formation. The proper execution of the normal silencing process has significant consequences for the human placenta. As described by Fowden, Coan, Angiolini, Burton, and Constancia (2011), imprinted genes "regulate the growth of many cell types within the placenta with consequences for the area and passive diffusion properties of the materno-fetal exchange surface." Imprinted genes alter the expression of transporters that control the movement of substances across the placenta. Because imprinted genes in the placenta are responsive to environmental information and can act as sensors, they contribute to the placental phenotype with consequences for programming effects on health and disease (Fowden, Forhead, Coan, & Burton, 2008). There are at least 100 known imprinted genes in mice but there may be more than 1000 and most of them are expressed in the placenta. It is not precisely known at this time, if or how imprinted genes in placental tissue regulate and control psychobiological stress signals between mother and fetus.

The HPA Axis Is Not Only Cortisol

Our research team has been exploring the effects of stress and specifically the HPA axis on developmental processes for over 40 years. Our early interest was, and continuing interest is, on the influences of early life exposure to stress-related hypothalamic releasing and inhibiting factors, peptides and hormones released from the pituitary gland, and molecules from target organs (Beckwith, Sandman, Hothersall, & Kastin, 1977; Champney, Sahley, & Sandman, 1976; Moldow, Kastin, Hollander, Coy, & Sandman, 1981; Sandman & Kastin, 1981; Sandman & O'Halloran, 1986; Sandman & Yessaian, 1986). Our initial studies demonstrated that multiple levels of the endocrine stress system exerted profound and persisting effects on the brain and behavior.

As reviewed in this chapter, during the past 20-plus years our group has been examining the effects of stress and activation of the HPA and placental axis on the human fetus. Our human fetal programming projects may be unique in the assessment of multiple levels of endocrine signals transmitted between mother and fetus. We have included in our strategy and analysis a novel approach to identify fetal stress exposure. Instead of relying *exclusively* on measures of maternal stress and *assuming* that the fetus has "experienced" the consequences, we consider variations in the levels of pCRH as *evidence* that the fetus has experienced and responded to a stress signal. Although extensive research supports the conclusion that pCRH is responsive to stress (Sirianni, Rehman, Carr, Parker, & Rainey, 2005) and that it is the stress index most consistently associated with birth outcomes, our studies are unique in including pCRH to predict neurodevelopmental outcomes. Thus, the focus on a biological marker of fetal exposure to stress, pCRH, capitalizes on a direct index of fetal experience and avoids the pitfalls associated with assuming that maternal stress results in a coherent and consistent maternal physiological profile that is transmitted to, and transduced by, the fetus.

Race/Ethnicity, Culture and Socioeconomic Status

One next step in moving the field forward will involve consideration of important modifiers or moderators of prenatal influences on developmental outcomes. Some have asserted that the development of culturally or ethnically specific models of prenatal stress influences may be necessary for a comprehensive understanding of early life influences on development (Hogue, Hoffman, & Hatch, 2001; Kramer, Goulet, Lydon, Seguin, & McNamara, 2001; Lu & Halfon, 2003; Misra, Guyer, & Allston, 2003). Consistent with this view, evidence from our collaborative work suggests that race/ethnicity, culture, and socioeconomic status represent theoretically and empirically important constructs, and that their influences are manifested is several distinct ways. First, in some cases, a particular psychological dimension is a meaningful predictor of outcomes, only for certain groups, or exerts more potent effects in one group compared to another. For example, perceived racism is a

predictor of lower birth weights among African American women but not White women (Dominguez et al., 2008) and higher levels of social support are predictive of larger birth weights among Latina women, but not among non-Hispanic White women (Campos et al., 2008). A second way that these demographic and cultural factors may be important to consider is in the context of potential biological mediators of stress on outcomes. We have demonstrated that among African American and Latina women, elevated cortisol is more likely to stimulate accelerations in the pCRH trajectory than among White women (Glynn, Dunkel-Schetter, Chicz-DeMet, Hobel, & Sandman, 2007). Further, Holzman, Jetton, Siler-Khodr, Fisher, and Rip (2001) have shown that the threshold of exposure to pCRH that results in a preterm birth is lower among African American women. Third, taking into account important demographic and cultural constructs may render it more possible to detect relations between indicators of psychological states and biological ones. For example, certain social support related buffers may only be beneficial among higher risk groups. Among African American women and women who have experienced socioeconomic disadvantage over the life course, communalism (a cultural orientation emphasizing interdependence) is associated with prenatal blood pressure profiles. This association is not detected among White and more affluent women (Abdou et al., 2010). Similarly, psychological distress is associated with blood pressure profiles across gestation among African American women, but not among White women (Hilmert et al., 2008).

Patterns and Trajectories

The physiological and psychological states of pregnancy and their interactions are complex and dynamic. There are significant knowledge gaps regarding the physiology of pregnancy and we are far from a comprehensive understanding of the influences of the perinatal period on women's psychological and cognitive functioning (Glynn & Sandman, 2011). Our conceptual models predict associations between prenatal psychological and physiological states and responses and that this linkage should reveal mediational pathways through which prenatal psychological distress or stress exposures influence offspring development. Perhaps, because of the complexity of human pregnancy and the present state of knowledge, it is not surprising that we have not been successful in validating these associations empirically. A relatively new conceptual perspective that we have taken is to characterize patterns or trajectories of our stress measures across gestation, in addition to assessing levels of psychological distress or biological stress at specified gestational intervals. We have coupled this conceptual shift with new analytical techniques that allow the examination of these trajectories and patterns of stress indicators (e.g., multilevel modeling and general growth mixture modeling) as predictors of outcome. As a result, we are confirming that patterns during gestation or development reveal relations between the psychological and biological constructs of interest and also are independent, and sometimes far superior predictors of birth and child outcomes (Davis & Sandman, 2010; Glynn et al., 2008; Kane et al., 2014; Sandman et al., 2012a, 2012b).

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Maternal Anxiety, Mindfulness, and Heart Rate Variability During Pregnancy Influence Fetal and Infant Development

14

Bea R.H. Van den Bergh

"The idea that at birth the child is an individual is becoming more and more accepted. Prenatally, this individual is already unique because of his unique genotype and because for the past nine months it underwent the influences of his specific prenatal intra -uterine environment." (Van den Bergh, 1981, p. XII)

Abstract

In this chapter, we present our recently conceptualized model on Developmental Origins of Behavior, Health, and Disease (DOBHaD) in which we incorporate the results of four of our studies as examples to demonstrate how each topic influenced the model; in addition, we provide a brief overview of relevant literature. The study of DOBHaD encompasses both, short- and long-term consequences of conditions in the environment relevant to behavior, health, and disease risk and addresses research issues related to the interface between developmental, behavioral, and medical science. In the first section, one early and one later study from the Leuven prospective follow-up project are described. Study 1 examines the influence of maternal emotions on fetal and neonatal behavioral state-related activity and on infant activity. Study 2 examines the relationship between fetal behavioral states and self-regulation in childhood and adolescence. In the second section, two recent studies from the Tilburg prospective follow-up project are described. Study 3 explores how variation

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in both negative emotions (i.e., maternal anxiety) and positive emotions (i.e., maternal mindfulness) influence infant neurocognitive development. Study 4 explores the issue of how exposure to a past, resolved maternal anxiety disorder influences maternal heart rate variability during pregnancy as well as infant heart rate variability, which in turn influences infant temperament. In the final section we summarize our results, use them to explain applications of the DOBHaD model, and speculate on potential clinical implications.

Keywords

Prenatal stress • Maternal anxiety during pregnancy • Maternal mindfulness during pregnancy • Infant event related potentials (ERPs) • Autonomic nervous system • Infant temperament • Fetal behavioral states • Self-regulation • Infant cognition • Heart rate variability in pregnant women • Infant heart rate variability

Introduction

To introduce his aggregated volume entitled *Prenatal determinants of behavior*, Joffe (1969, p. ix) wrote: "This book is an attempt to gather together from diverse sources the research which relates events prior to birth to effects on the postnatal behavior of organisms. Such studies are an extension of the widespread and intensive interests in the behavioral effects of events in the early environment to the organism's earliest environment—the prenatal environment. It is hoped that bringing together studies which arose from a variety of experimental interests and appeared in a wide range of publications will stimulate further interest in a field which only now is being delineated as a research area in its own right." When he wrote these words, one wonders whether Joffe could have foreseen how much interest the field of prenatal environmental influences was going to gain in the period that lay ahead. Joffe qualified his introductory sentences by stating that, over the centuries, there had been a fluctuation in the belief of prenatal influences and that apparently the status of the area did not depend so much on the available evidence as on the prevailing climate or opinion: "Medical opinion appears to have accepted the general proposition at times and ridiculed at others" (Joffe, 1969, p. 1). Likewise Stott (1958, p. 42) mentioned that effects of psychosomatic stress during pregnancy was "a topic which has fallen under the taboo of "old wives tales." Although the general proposition of prenatal influences is now widely accepted, the underlying mechanisms of how the prenatal environment influences the developing organism and modulates brain structure–function relations, behavior, health, and disease risk are yet to be fully elucidated (e.g., Crews et al., 2012; Fox, Levitt, & Nelson, 2010; Hofer, 2014; Kolb et al., 2012; Lutz & Turecki, 2014; McEwen & Morrison, 2013; Meaney, 2010; O'Connor, Monk, & Fitelson, 2014; Reul et al., 2015; Schlotz, Jones, Godfrey, & Phillips, 2008; Zannas & West, 2014). Debates on which theoretical frameworks are best able to integrate most of the available research results in a coherent way are ongoing (e.g., Bock, Poeschel et al., 2014; Bock, Rether, Gröger, Xie, & Braun, 2014; Daskalakis, Bagot, Parker, Vinkers, & de Kloet, 2013; Daskalakis & Yehuda, 2014; Del Giudice, 2012; Del Giudice, Ellis, & Shirtcliff, 2011; Hanson & Gluckman, 2014; Lee & Goto, 2013; Lupien, McEwen, Gunnar, & Heim, 2009; Nederhof & Schmidt, 2012; Ortega-Martínez, 2015; Schlotz & Phillips, 2009).

An important reason for our scientific quest, which started some 35 years ago, was the question of when and how individual differences between people arise as well as when and how

these processes can be studied. In common with Joffe (1969), we extended our search to the organism's earliest environment and started to examine such issues as the variation in maternal emotional state during pregnancy and how this might lead to a variation in the behavior of the offspring before and after birth, including potential behavioral problems. To achieve this goal, concepts and methods were borrowed from the fields of developmental psychobiology/developmental behavioral neuroscience (e.g., Blumberg, Freeman, & Robinson, 2010; Gottlieb, 1997; Kolb et al., 2012; Lickliter, 2007; Michel & Moore, 1995), developmental cognitive neuroscience (Johnson, 2011; Johnson & de Haan, 2011), developmental affective neuroscience (e.g., Pollak, 2005; Schechter, 2012), developmental psychology (e.g., Kopp, 1982; Rothbart & Derryberry, 1981; Sameroff, 1975; Sameroff & Chandler, 1975), clinical psychology, and child psychiatry (e.g., Bayley, 2006; Rutter, 1987, 1995, 2002; Verhulst, van der Ende, & Koot, 1996). Our studies contributed to the efforts of interdisciplinary and multidisciplinary researchers examining the Developmental Origins of Health and Disease (DOHaD) hypothesis. This hypothesis originated from the "fetal programming of adult disease"—the hypothesis which states that an adverse fetal environment induces plastic responses that increase the risk of chronic diseases such as type 2 diabetes and coronary heart disease later in life (Barker, 1990, 1995, 2004; Barker & Osmond, 1986). Studies testing the DOHaD hypothesis include early prenatal and perinatal origins of a wide range of diseases and disorders, physical as well as mental, by adverse influences during sensitive periods of development (Gillman, 2005; Gluckman & Hanson, 2004; Gluckman, Hanson, & Beedle, 2007; Hanson & Gluckman, 2014; Nathanielsz, 1999; Meaney, 2010; Meaney, Szyf, & Seckl, 2007; Phillips & Jones, 2006; Seckl, 2007; Seckl & Holmes, 2007; Schlotz et al., 2008). The DOHaD research field was influenced by researchers studying the adaptive and/or maladaptive nature of neural, physiological, and behavioral responses to environmental stressors. Specifically, it was influenced by early life stress (ELS) research, targeting consequences of ELS and individual differences in resilience and vulnerability to stress and adversity later in life and the development of stress-related diseases (e.g., de Kloet, Claessens, & Kentrop, 2014; de Kloet, Joëls, & Holsboer, 2005; de Kloet, Karst, & Joëls, 2008; Gunnar & Quevedo, 2007; Heim & Nemeroff, 2001; Koolhaas et al., 2011; Lupien et al. 2009; McEwen & Morrison, 2013; Meaney, 2010; Meaney, Szyf, & Seckl, 2007; Reul et al., 2015; Swaab, Bao, & Lucassen, 2005). Most of this work demonstrated support for a "three-hit model" (Daskalakis et al., 2013). This is a model in which early life adversity does not directly or inevitably lead to disorder or disease but rather genetic factors (hit 1) in interaction with early life environmental inputs and experience-related factors (hit 2) lead to a certain phenotype with differential susceptibility to later-life challenges. In an individual with a certain phenotype when exposed to a later-life environment (hit-3), his or her mental functions may become compromised and a higher risk of psychiatric symptoms may arise (vulnerability); but when exposed to another type of environment the same individual is expected to be resistant to mental dysfunction (resilience) (Daskalakis et al., 2013, p. 1867).

Recently, we introduced a model to describe adaptation to environments in general rather than limited to early adversity (Van den Bergh, 2010, 2011a, b). Our model is compatible with the latter models (e.g., Daskalakis et al., 2013) in that it attempts to explain the association between early life events (typical as well as atypical; positive as well as negative) and physical and mental health as well as physical and mental health problems in a schematic way. Our model extended the concept of DOHaD by explicitly incorporating the study of behavior and brain-behavior relations in the DOHaD hypothesis (Van den Bergh, 2010, 2011a, b; see Fig. 14.1). The study of the Developmental Origins of *Behavior*, Health and Disease (DOBHaD) encompasses both short- and long-term consequences of conditions in the environment relevant to behavior, health, and disease risk and addresses research issues related to the interface between developmental, behavioral and medical science.

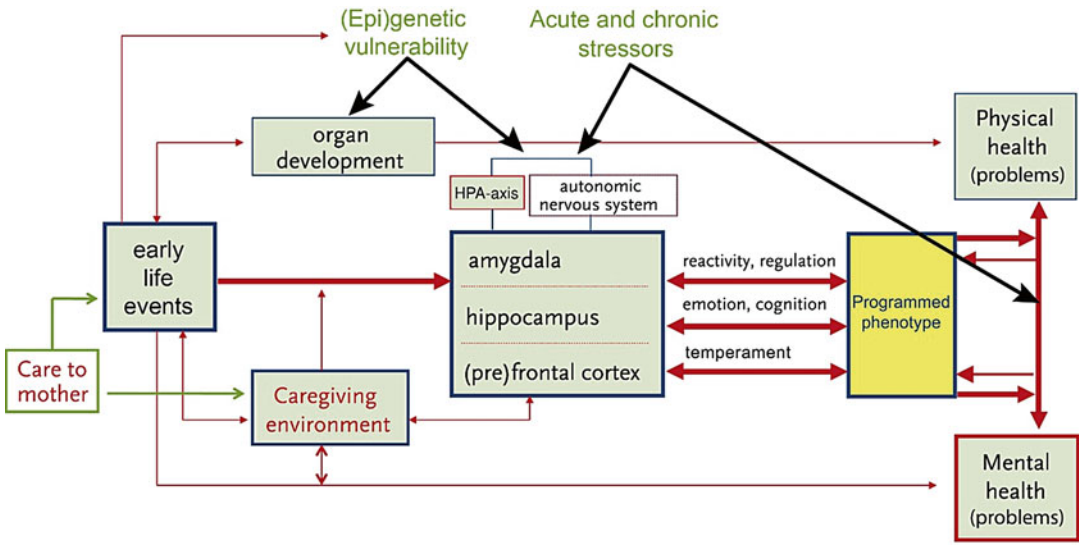

Fig. 14.1 Developmental origins of early brain and behavior development, mental health and mental health problems (adapted from Van den Bergh, 2010, 2011a, b)

As demonstrated in this chapter, our DOBHaD model can be used to integrate both new and older data. It integrates the results of human literature on prenatal stress and relates them to results of preclinical, experimental animal studies which associated offspring outcome measures to changes in underlying neural circuits and causally related epigenetic processes (Bock, Poeschel et al., 2014; Bock, Rether et al., 2014; Weaver et al., 2004). The model illustrates that early life events will influence the development of organs, such as the heart, lungs, bones, kidneys and including development of the brain and neural circuitry, influence behavior and evolve into a certain programmed phenotype that finally leads to mental and physical health problems in some later-life environments and to mental and physical health in other environments.

During the prenatal and early postnatal life period the brain is subject to dramatic developmental processes; this period represents a phase of high susceptibility towards environmental influences. The specific physiological, neuroendocrine, and metabolic alterations that enable the individual to adapt to its early environment depend on the timing, duration, type, and magnitude of exposure of the organism to environmental factors and also are influenced by the individual's genetic and epigenetic susceptibility. The latter implies that individuals differ in their susceptibility to early environmental factors (Daskalidas et al., 2013, Daskalakis & Yehuda, 2014; de Kloet et al., 2014; Nederhof & Schmidt, 2012). A causal pathway often studied in animal research is the alteration of the programming of the stress-regulating system [including epigenetic modifications in the hypothalamic–pituitary–adrenocortical (HPA)-axis and autonomic nervous system] by early events. Adaptive developmental plasticity leads to changes in the limbic brain structures (hippocampus, amygdala) and the prefrontal cortex, which are involved in (stress) reactivity and regulation patterns, in emotional (e.g., anxiety, anger) and cognitive (e.g., sound perception, appraisal, learning, memory) processing and in temperamental variation in behavior (e.g., fearfulness or negative reactivity; surgency or positive reactivity; harm avoidance,

novelty seeking) (Kolb et al., 2012). These changes in underlying circuits may influence how an individual "behaves" (i.e., senses, perceives, appraises, responds) in its environment in general. Moreover, situations of acute and chronic stress and adversity later in life will trigger its stress system in a particular way (Ladd et al., 1999; Lee & Goto, 2013; Seckl, 2007) and express the evolving "programmed phenotype." Exposure of a person with a certain programmed phenotype to some types of environments may lead to behavioral problems, psychopathology, or more generally mental health problems (vulnerability) while the same phenotype exposed to another type of environment may lead to mental health or resistance to mental dysfunction (resilience).

Having developed a general model of adaptation to the early environment, the focus of this chapter is to demonstrate how research findings from our laboratory over 35 years were integrated into the model, describing four studies in particular which serve as exemplars (Braeken et al., 2014; Van den Bergh, 1989, 1990; Van den Bergh & Mulder, 2012; van den Heuvel, Donkers, Winkler, Otte, & Van den Bergh, 2014). Collectively, the research questions in the four studies focused on psychological and physiological processes and their interplay in examining individual differences in behavior in the offspring. Specifically, these longitudinal studies focused on describing neurobehavioral (Studies 1, 2, and 4), neurocognitive (Study 3), and neurophysiological (Studies 3 and 4) functioning from fetuses (Study 1) to infants (Studies 3 and 4) to adolescents (Study 2). It is important to note that, although all of the studies involved uncomplicated pregnancies in healthy pregnant women, there were a full range of both anxiety and mindfulness scores on the instruments used to measure these maternal variables (e.g., 25 % of women were highly anxious (Study 1); some women had a resolved anxiety disorder (Study 4). To show advances in research thinking and techniques over time, the chapter is divided into two major sections. In the first section, early and later studies from the Leuven prospective follow-up project[1] are described (Van den Bergh, 1989, 1990; Van den Bergh & Mulder, 2012). Study 1 examines the influence of maternal emotions on fetal and neonatal behavioral state-related activity and on infant activity (Van den Bergh, 1989, 1990, 1992; Van den Bergh et al., 1989). Study 2 examines the relationship between fetal behavioral states and self-regulation in childhood and adolescence (Van den Bergh & Mulder, 2012). Because other results of the Leuven prospective follow-up project are reviewed elsewhere (see Van den Bergh, 2011b; Van den Bergh, Loomans, & Mennes, 2015), they were not included here. In the second section, recent studies from the Tilburg prospective follow-up project are described. Study 3 explores how variation in both negative emotions (i.e., maternal anxiety) and positive emotions (i.e., maternal mindfulness) influence infant neurocognitive development (van den Heuvel et al., 2014). Study 4, explores the issue of how exposure to a past, resolved maternal anxiety disorder (what is presumed to be an atypical maternal emotional state) influences maternal heart rate variability during pregnancy as well as infant heart rate variability, which in turn influences infant temperament (Braeken et al., 2013). In the final section, we summarize how exposure to maternal emotional state during pregnancy may have changed offspring behavior (i.e., his or her sensation, perception, appraisal, reactivity), use

[1] The Leuven project was started in 1986 and examined the link between maternal anxiety during pregnancy and offspring development (i.e., fetal behavioral states and postnatal neurocognitive, behavioral and/or emotional development at the ages of 1 and 10 weeks, 7 months, and 8–9, 14–15, 17, and 20 years of age, e.g., Mennes, Stiers, Lagae, & Van den Bergh, 2006; Mennes, Van den Bergh, Lagae, & Stiers, 2009; Van den Bergh & Marcoen, 2004; Van den Bergh, Mennes, et al., 2005; Van den Bergh, Mulder, et al., 2005; Van den Bergh et al, 2008). Subsequently, the Tilburg prospective project was started in 2010 as part of the Prenatal Early Life Stress (PELS)-project (see section "Maternal Heart Rate Variability and Emotions Are Associated with Infant Cognition and Heart Rate Variability") and aimed to examine the link between both negative and positive maternal emotions during pregnancy and offspring outcome.

these results to explain the potential use of the DOBHaD model and speculate on potential clinical implications.

Section 1: Maternal Emotions, Fetal and Neonatal Behavioral States, and Child and Adolescent Self-Regulation

Historical Background

In the 1970s, the introduction of real-time ultrasound imaging enabled the direct, standardized, noninvasive study of the human fetus in utero. There was a resurgence of the work of early scientists who attempted to demonstrate classical conditioning (Ray, 1932; Spelt, 1948) and habituation (Peiper, 1925; Sontag & Wallace, 1934) in the fetus and those who examined whether maternal emotions influenced fetal behavior (Sontag, 1941, 1944). As knowledge that had been gained in various fields was brought together and reexamined with new techniques (Eskes, 1992), new paradigms emerged. Groundbreaking insights were reached, such as those on the developmental sequence of fetal movements, starting from 7.5 weeks gestational age (de Vries, Visser, & Prechtl, 1982, 1985) and on the development of fetal behavioral states (Nijhuis, Prechtl, Martin, & Bots, 1982).

Reviewing the literature a decade following this initial surge of research, Hepper (1992) suggested that the renewed interest in the fetal period may have arisen because of a change in the view of the capabilities of the newborn from being poorly developed and unable to adjust to his/her environment to having abilities exquisitely enabling adaptation. He attributed this reappraisal to the ability of scientists to ask the right questions. His ideas were reminiscent of Joffe's (1969) interpretation of the importance of the prevailing climate or opinion regarding prenatal influences. In the same vein, Prechtl remarked that some writings of fetal behavior from around the turn of the nineteenth to twentieth century "were surprisingly modern, while writings from the 1920s and 1930 often seem extremely limited or obsolete"; the dominance of reflexology and behaviorism during the latter period may account for the prevailing view at that time (Prechtl, 1984, p. 5). One of the clear implications of psychobiological research being (re)-focused on the competences of the neonate and fetus was "the importance of early events in shaping subsequent development" (Smotherman & Robinson, 1995, p. 15), the study of which became a major topic of our research program.

We began our own empirical research program with an analysis and synthesis of the diverse literature on human maternal emotions during pregnancy (Van den Bergh, 1981, 1983, 1989). Many of the studies reviewed showed methodological shortcomings, such as: a failure, to specify the sample characteristics; insufficient control conditions; conclusions based on retrospectively obtained, inadequate assessment of predictor and outcome variables; lack of sound statistical methods; the problem of causation versus correlation; and the problem of rater bias, when the mother completed self-report questionnaires on her own emotional state or her offspring's behavioral problems. Nevertheless, already at that time, results of the methodologically sound studies (e.g., Farber, Vaughn & Egeland, 1981 and see Carlson & Labarba, 1979 for a review) led to one general conclusion: negative maternal emotions during pregnancy may influence prenatal as well as postnatal behavior in the offspring and lead to behavioral problems and diseases later in life. Indeed, there was evidence that increased levels of negative emotionality and stress in the pregnant women may influence fetal brain development and behavior such as that shown by increased fetal heart rate (FHR) and motility (e.g., Copher & Huber, 1967; Sontag, 1941, 1966) and are associated with pregnancy and birth complications such as hyperemis gravidarum, toxemia, premature birth, and lowered birth weight (e.g., McDonald, 1968). Both negative maternal emotions and pregnancy and birth complications were shown to be associated with developmental irregularities and behavioral problems (e.g., Carlson & Labarba, 1979; Dörner, 1974; Erickson, 1971, 1976a, 1976b; Ferreira, 1960, 1965; Istvan, 1986; Knobloch & Pasamanick, 1966; Pasamanick & Knobloch, 1966; Pasamanick, Rogers, & Lilienfield, 1956; Sameroff & Zax,

1973; Sameroff & Chandler, 1975; Stott, 1958), altered mother–child interactions and adjustment (e.g., Davids & Holden, 1970; Davids, Holden, & Gray, 1963; Farber et al., 1981), childhood diseases (Stott, 1973; Stott & Latchford, 1976), and alterations in adult personality (Sontag, 1966). (Note: The link between maternal stress during pregnancy and childhood disease was recently confirmed in a very large scale ($n = 66{,}203$) prospective cohort study in Denmark (Tegethoff, Greene, Olsen, Schafner, & Meinlschmidt, 2012).

It was our aim to study processes that constitute offspring behavior in a prospective and standardized way as this would enable us to identify potential indices [or (bio)markers] of altered programmed phenotype and underlying mechanisms. Identifying indices and mechanisms is interesting from a basic scientific as well as from an applied, clinical point of view, namely to predict the risk of behavioral problems or disease in some environments later in life and, more generally, to set up innovative preventative and intervention strategies.

Study 1: Relationship Between Maternal Emotions and Fetal, Newborn, and Infant Behavior

In the early 1980s our research aims were to study two links which, according to our review, had not been systematically explored with a sound method, design or statistical technique: Can the influence of maternal emotions upon fetal behavior be established in the prenatal period? (first aim) and, is the prenatal influence established in the prenatal period reflected in the neonatal and infant behavior? (second aim)

While previous studies relied on maternal report of fetal movements or on fetal heart rate measures, the introduction of ultrasound in human research facilitated the study of these two links by enabling the direct measurement of fetal behavior for prolonged periods in pregnant women with varying levels of anxiety. Given the groundbreaking results from the work of colleagues in Groningen (de Vries et al., 1982, 1985; Prechtl, 1974, 1984; Visser, Poelman-Weesjes, Cohen, & Bekedam, 1987) and Leuven (e.g.,Casaer, 1979, 1993; Casaer & Devlieger, 1984; Casaer & Eggermont, 1985; Casaer, O'Brien, & Precht, 1973; Deprest et al., 1998; Van Assche, 1997; Van Assche, Holemans, & Aerts, 2001; Vandenberghe & Dewolf, 1990), we selected fetal and neonatal behavioral states and state-dependent movements as primary outcome variables. Fetal behavioral states emerge during the third trimester of pregnancy. They involve multiple interconnected neuronal networks. Functional (re)organization of sleep cycling likely occurs around 28–30 weeks gestational age (GA), 36 weeks GA, and 2 months of age (Nijhuis et al., 1999; Scher, 2008; Visser et al., 1987). From 36 weeks of gestation onward, the low-risk fetus exhibits two states of sleep and two states of wakefulness. (See Chap. 6 by Nijhuis this volume for a description of each state.) Fetuses typically pass through sleep cycles of non-REM (quiet) sleep and REM (active) sleep, lasting about 70–90 min (Visser, Mulder, & Prechtl, 1992). The time spent in wakefulness is usually less than 10 %. Typical fetal sleep states show a concordant (uninterrupted) association between the state parameters for a prolonged time and a simultaneous (synchronized) change of state parameters ($\leq$3 min) at their beginning and end (transitions). The degree of sleep state stability and the duration of transitions into and out of a particular state are considered measures of neurophysiological development, integrity, and maturity (Mulder, Morssink, Van Der Schee, & Visser, 1998; Visser et al., 1992).

Our study was designed to examine both the issue of the influence of maternal emotions on fetal behavior (aim 1) and the effects of alterations in fetal behavior on neonatal and infant behavior (aim 2) in the same population. The sample included 86 Dutch women, 18–30 years of age, in their first pregnancy which was singleton, low-risk, with no medication or drug use. All pregnancies were dated using the last menstrual period and/or an ultrasonographic examination before 14 weeks. The course of pregnancy remained unremarkable with delivery from 36 to 41 weeks of gestation. All infants had a birth weight above the 10th percentile, a 5-min Apgar score of 9 or 10, and no postnatal medical complications while in hospital. Maternal pregnancy anxiety was measured at 12–22, 23–31, and

32–40 weeks GA and at 1 week, 10 weeks, and 7 months after delivery using the self-report state anxiety subscale of the Spielberger State Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970; Van der Ploeg & Defares, 1980). In a subsample of 37 women [Mean age (SD) = 26.6 (2.55)], simultaneous recordings of fetal heart rate (FHR), fetal generalized body movements (GM) and fetal rapid eye movements (REM) were made continuously for 2 h at 36–38 weeks GA. FHR recordings were collected using a cardiotocograph and scored visually into episodes of heart rate pattern (HRP) A, B, C, or D (Nijhuis et al., 1982). Fetal generalized body and eye movements were observed and video-recorded using real-time ultrasound scan. The presence of states (or coincidence) 1F–4F was identified according to predefined criteria (see Mulder, Visser, Bekedam, & Prechtl, 1987; Nijhuis et al., 1982). For some analyses, the data of state 2F and 4F, were combined (in order to have one measure of states in which fetuses are actively making movements); in other ones, state 2F, 3F, and 4F were combined to have one measure of state other than 1F/quiet sleep. On day 5–6 after birth, a comparable 2-h observation was carried out on the newborn. At 7 months of age, maternal report of the infant activity was dichotomized as problematic or not problematic. To examine our two research aims, we constructed and tested several sets of nested linear structural relation (LISREL)-models, including state and/or trait anxiety in each pregnancy trimester as predictor variable(s). To select the fetal behavioral measures to be included in the LISREL-models, component analyses were performed, with varia-max rotation of components with eigenvalue >1 and the measures with the highest values on each of the components were selected; comparable neonatal measures were then introduced in the models. Fetal sex was introduced as a second predictor variable since our data showed that male fetuses were more active than female fetuses. Maternal anxiety postpartum had no significant association with neonatal behavioral measures and was not introduced in the neonatal LISREL model. However, as maternal anxiety 7 months after delivery was significantly associated with the infant activity measure, we introduced it as a second predictor to examine the link with infant activity at 7 months. The models were tested on $n = 28$ fetuses/neonates for whom all data were available.

As can be seen in Fig. 14.2—Model A, LISREL modelling showed that fetuses of high anxious mothers made more general movements and head movements and that male fetuses made more general movements than females. (Note: Only the final model of nested LISREL models are shown.) The prenatal influence of maternal State anxiety also was reflected in neonatal behavior; infants who made more head movements as fetuses, made more general movements and more head movements as neonates. The observation that the percentage of fetal head movements (rather than the percentage of fetal general movements) was significantly related to neonatal head and general movements may indicate that the newborns had difficulties in adjusting to gravidity, which may have more effect on making body movements than on making head movements (Michel & Moore, 1995; Prechtl, 1984). Model B illustrates that maternal Trait anxiety had a negative influence on the mean duration of epochs of coincidence of fetal State 1, and that infants who have shorter epochs of State 1F as fetuses also have shorter duration of epochs of State 1 as neonates. Model C reveals that at 7 months after birth, maternal anxiety during pregnancy had an indirect effect on activity level of the infant (i.e., via influencing fetal general movements) and maternal anxiety measured at 7 months after delivery had a direct influence on infant activity level. By this time, the mother and, infant would have had much more opportunity to interact than at four or five days after birth. Of course, these differences may have resulted because infant activity was reported by the mother and reflect her bias. In future research, direct observation of maternal–infant interactions at this age might be useful in untangling these effects.

In summary, the LISREL modelling, together with other results from this cohort (e.g., those

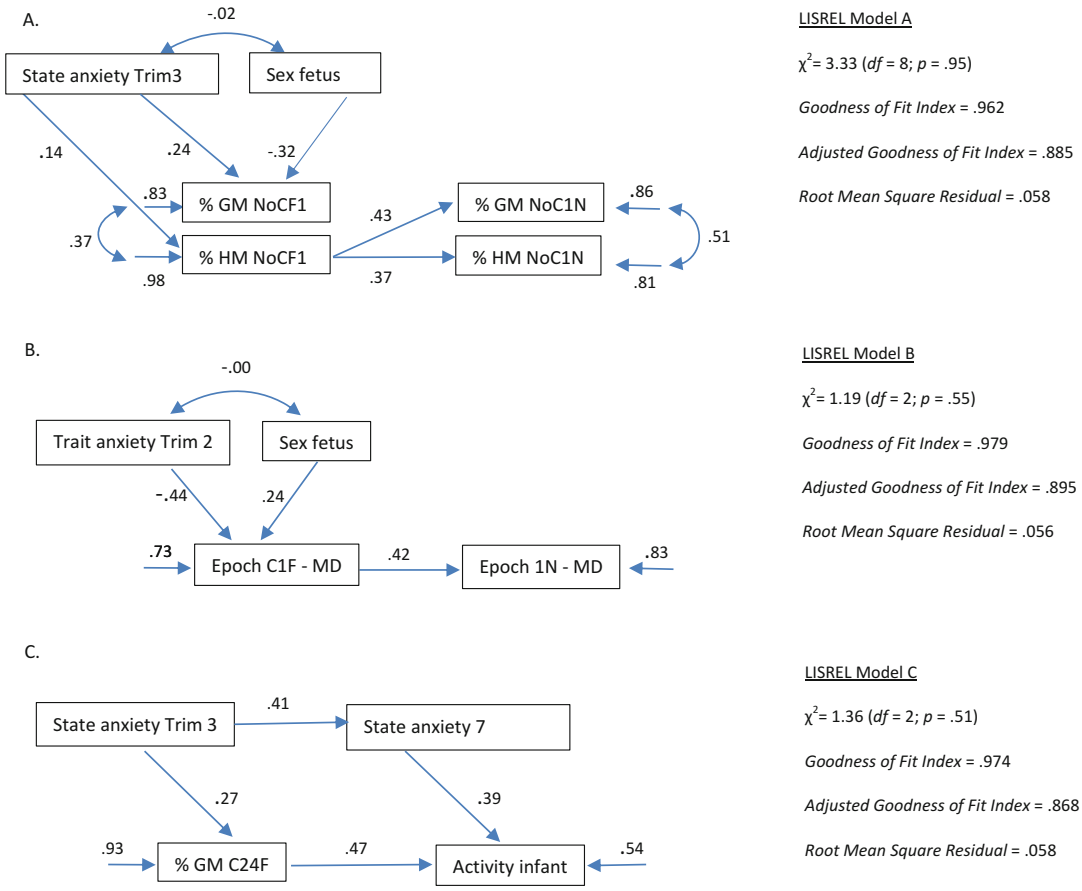

Fig. 14.2 LISREL model A,B,C concerning the relation between maternal anxiety during pregnancy, fetal and neonatal behavioral states and motor activity, and infant activity (adapted and translated from Van den Bergh, 1989). Legend: 7 months = 7 months after delivery; df = degrees of freedom; Epoch C1F-MD: Mean duration of epochs of coincidence State 1 in the fetus; Epoch 1N-MD: Mean duration of epochs of State 1 in the neonate; % GM = percentage of general movements; % HM = percentage of head movements; NoC1F = epochs of coincidence other than State 1F; C24F = epochs of coincidence 2F and 4F; Trim2 = Second trimester of pregnancy; Trim3 = Third trimester of pregnancy

showing that infants of high anxious pregnant mothers had a more difficult temperament at 10 weeks and cried more, were hungrier, had more stomach cramps at 7 months than infants of mothers who were less anxious, see Van den Bergh, 1989, 1990, 1992) answered our two research aims cited above in a positive way. Results of Study 1 can be integrated into our DOBHaD model (see Fig. 14.1) in the following way: prenatal exposure to maternal anxiety (early life events) in interaction with supposed (epi)genetic factors, may have an enduring influence on (or programs) fetal/neonatal brain development and behavioral functioning as reflected in higher fetal and neonatal reactivity. This higher reactivity may evolve into a programmed phenotype including seeking out arousal-inducing events. Moreover, the induced behavioral alterations observed in the offspring of mother's with high anxiety levels may influence the quality of the interaction between mother and child (i.e., the caregiving environment) in a negative way, increasing the risk of subsequent offspring behavioral or mental

health problems. [Further explanation of the (use of the) DOBHaD model is given in the final section which clarifies the integration of the results of this study.]

A link between the level of stress and anxiety of the mother during pregnancy and ultrasonographically observed fetal behavior and fetal heart rate is now well established (for a review see DiPietro, Costigan, Pressman, & Doussard-Roosevelt, 2000; Kafalí, Derbent, Keskí, Símavlí, & Gözdemír, 2011; Monk et al., 2011; Van den Bergh, Mulder, Mennes, & Glover, 2005). Most studies have reported that increased maternal anxiety was associated with increased fetal arousal/wakefulness and increased FHR variability and % of body movements during states 2F and 4F. As an example, DiPietro and colleagues (2000) observed that fetuses of women with a positive versus negative attitude toward pregnancy exhibited different overall levels of motor activity (reduced versus increased, respectively). Although positive (pleasant, optimistic) emotions and negative stressors are believed to be regulated by the same physiological system [hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system (ANS)], the negative emotions may have reflected chronic negative conditions, which were both unpredictable and uncontrollable and triggered a stress response, involving cortisol release (Koolhaas et al., 2011), while positive emotions (dispositional optimism) have been linked to lower levels of cortisol responses under stress (Jobin, Wrosch, & Scheier, 2014).

Study 2: Relationship Between Fetal Behavioral State and Child and Adolescent Self-Regulation

Having observed in Study 1 that maternal anxiety did influence fetal behavioral state and was associated with alterations in state-related fetal activity level, we turned our attention to the influence of fetal states on self-regulation. Specifically, we addressed the question: Is fetal behavioral state organization a biological precursor of child and adolescent self-regulation? (Van den Bergh & Mulder, 2012)

Background

Sleep plays a critical role in early brain development, arousal regulation, attention and cognition (Graven & Browne, 2008; Mirmiran, Maas, & Ariagno, 2003; Mulder, Ververs, de Heus, & Visser, 2011; Peirano, Algarín, & Uauy, 2003) and the study of sleep ontogeny (i.e., behavioral state organization) can be used to identify patterns of brain maturation (Scher, 2008). For example, in one study (Scher, Steppe, & Banks, 1996), sleep measures of both the healthy preterm infant (assessed at term equivalent age) and the healthy full-term newborn were predictive of performance on the Bayley scales of mental development at 12 and 24 months. In another study (Holditch-Davis & Edwards, 1998), in high-risk premature infants born at gestational ages from 27 to 29 weeks onwards, the degree of sleep state control after birth was associated with postnatal neurodevelopmental status at term equivalent age. The predictive value of these measures for behavioral developmental outcome in later life has remained unexplored due to a lack of long-term follow-up studies. Therefore, in a nonclinical sample (see Study 1 for a description of this sample), we examined whether differences in sleep state organization in the near term fetus could account for differences in child and adolescent self-regulation (Van den Bergh & Mulder, 2012).

Theories of self-regulation presume that humans, from prenatal life or birth onward, display individual differences in reactivity and regulation that have implications for subsequent development and adaptation (Calkins & Fox, 2002; Gunnar, Talge, & Herrera, 2009; Henrichs & Van den Bergh, 2015; Kochanska, Coy, & Murray, 2001; Kopp, 1982; Posner & Rothbart, 2000; Pruessner et al., 2010). Reactivity is understood as the arousability of physiological and behavioral systems, while self-regulation refers to neural and behavioral processes which function to modulate this reactivity. Interestingly, in some theories, temperament has been defined as constitutionally based individual differences in reactivity and regulation (Rothbart & Ahadi, 1994; Rothbart & Bates, 1998; Rothbart & Derryberry, 1981; Rothbart, Sheese, Rueda, & Posner, 2011). As the infant and child mature,

later-developing neural structures become integrated into the existing neural organization, which involves reorganization of circuits (Michel & Moore, 1995). Due to this patterned reorganization, initial reactive forms of regulation are supplemented by an increasing capacity for volitional, effortful control or self-regulation (Derryberry & Rothbart, 1997). Much of the self-regulation development results from increasing volitional control over attentional processes and enhanced inhibitory control over motor behavior (Calkins & Fox, 2002). Starting in childhood and continuing throughout adolescence, executive functions such as attentional focusing, maintenance and shift of focusing, and inhibitory control become integrated into complex emotional and behavioral regulatory processes. These processes, in turn, are involved in planning and goal setting, responsible decision making, emotional and motivational changes, and interpersonal relationships (Nelson et al., 2002; Rothbart & Bates, 1998; Van den Bergh & Mulder, 2012, p. 585). Failure of self-regulation in one way or another is a characteristic feature of behavioral problems and mental disorders (Henrichs & Van den Bergh, 2015).

At the time of our study, we could find no empirical work on individual differences in typical fetal brain maturation processes, such as expressed in fetal behavioral state organization or in relation to the long-term consequences for self-regulation. Thus, the aim of this prospective longitudinal study was to examine which measures of fetal behavioral state organization in the normal, near-term fetus are predictors of measures of self-regulation obtained from the same individuals when 8–9 and 14–15 years of age.

A total of 73/86 offspring participated in this second study. Twenty-five mother–offspring pairs who had participated in the fetal observation part of the Leuven study detailed above and had complete data for both the fetal behavioral observation session at the end of pregnancy and a follow-up study on the offspring at ages 8–9 or 14–15 were included. The reference (i.e., comparison) group consisted of 48 mothers and their, children/adolescents who participated only in the follow-up study but not in the fetal observation study.

For the follow-up study reported here, the mothers completed Dutch versions of temperament questionnaires, measuring concepts of reactivity (i.e., positive reactivity (or surgency) and negative reactivity) and of self-regulation (i.e., effortful control). The Children's Behavior Questionnaire (CBQ; Ahadi, Rothbart, & Ye, 1993; Rothbart, Ahadi, Hershey, & Fisher, 2001 translated and validated for a Dutch-speaking sample by Van den Bergh & Ackx, 2003) was used when their children were 8–9 years of age and the revised Early Adolescence Temperament Questionnaire (EATQ-R; Capaldi & Rothbart, 1992; Ellis & Rothbart, 2001; translated and validated for a Dutch-speaking sample by Hartman, Oldehinkel, De Winter, & Ormel, 2002) when the children were 14–15 years old. Only the temperament data concerning self-regulation are used in this study. Statistical modelling of the fetal–child–adolescent data demonstrated that one behavioral state measure, namely the time a typically developing fetus takes to pass from quiet sleep (S1F) to active sleep (S2F) in the last month before birth, is associated with her/his degree of self-regulation in childhood and adolescence. In particular, fetuses exhibiting sharp, synchronous transitions from quiet sleep into active sleep, compared with fetuses showing non-synchronized transitions (lasting >3 min) reached a higher level of effortful control (i.e., higher than the reference group but within normal ranges) both at 8–9 years and 14–15 years. Although the mechanisms underlying fetal state transitions are yet unknown and in need of future study, our results demonstrate that studies of sleep ontogeny can provide insights into fetal brain maturational processes which have implications for later environmental adaptation as well as developmental consequences for behavior. The results of Study 2 can be integrated in our DOBHaD model (Fig. 14.1). The supposed interaction between fetal environmental and (epi)genetic factors is reflected in synchronous fetal state transitions from quiet into active sleep in some fetuses and in asynchronous ones in other fetuses; these types of transitions are one element of early brain-behavior processes. These early differences may evolve into a programmed phenotype implying

optimal self-regulation in the former group and implying suboptimal self-regulation in the latter ones.

Section 2: Maternal Heart Rate Variability and Emotions Are Associated with Infant Cognition and Heart Rate Variability

Background

While the participants in the following studies were pregnant women and their offspring, the offspring were only measured after birth; however, the studies are included here because they have implications for fetal psychobiological development. The studies described below were part of the Prenatal Early Life Stress (PELS)-project, a multinational, European (i.e., Belgium, Netherlands, UK) project. The PELS-project was one of four projects of the "Stress and Mental Health" program (EuroSTRESS) in which the research questions aimed at increasing our knowledge of the basic mechanisms of stress-related mental disorders as well as advancing our understanding of how early life experiences, genetic makeup, and repeated traumatic events in adulthood might predispose a person to the development of mental health disorders. The ultimate objective of the EuroSTRESS-project was the use of this knowledge for the development of new treatment strategies and the prevention and/or amelioration of such disorders (for more information see: http://www.esf.org/coordinating-research/eurocores/completed-programmes/eurostress.html).

To identify specific maternal risk (and resiliency) factors during pregnancy having an influence on offspring neurodevelopment, a total of 151, 170, 190 pregnant women was recruited in the UK, Belgium and the Netherlands respectively. Each country gathered information on stress, anxiety and depression levels by having the pregnant women complete self-report questionnaires in addition to providing saliva samples for cortisol measures in each pregnancy trimester and at 2–4 and 9–12 months after delivery. For the infants, birth outcome data were collected; infant saliva cortisol was measured at 2–4 and 10–12 months-of-age. The Bayley Scales of Infant Development as well as a behavioral inhibition task were administered at 9–12 months after birth. In the Netherlands, infant event related potentials (ERP) and heart rate variability were measured at 2–4 and 9 months of age. Epigenetic analyses limited to one cohort (Belgium) showed that prenatal maternal emotional state, particularly pregnancy related anxiety, was associated with the methylation state of the NR3C1 gene in the child (Hompes et al., 2013; Hompes, 2014).

Study 3: Maternal Anxiety and Mindfulness During Pregnancy and Infant Neurocognitive Function

Electroencephalography (EEG), in general and event-related brain potentials (ERP) specifically, are unique tools which can be employed to assess cognitive functions such as attention, habituation and memory in early infancy. Indeed, ERP recordings in infants and the analysis of the responses have become well developed in the past 25 years (e.g., Alho, Sainio, Sajaniemi, Reinikainen, & Näätänen, 1990; for a review, see Kushnerenko, Van den Bergh, & Winkler, 2013). Cognitive abilities may be shown through infant responses to auditory stimuli (e.g., such as those used in auditory oddball paradigms) that mimic important features of the postnatal environment (Smotherman & Robinson, 1995). We examined how exposure to variation in maternal emotional state during pregnancy might influence the early neurocognitive development of the offspring using auditory stimuli, basing our studies on the work of Winkler and collaborators (Winkler, 2007; Winkler, Háden, Ladinig, Sziller, & Honing, 2009; Winkler et al., 2003).

Auditory attention is a key aspect of early neurocognitive function as it is a prerequisite of important skills, such as learning to speak and communicate with others. Moreover, some non-speech sounds also require one's attention because they may signal an opportunity or some danger and need further processing. Some sounds may be irrelevant for the current behavioral goals

and their processing should be stopped or suppressed (Kushnerenko et al., 2013). Obviously, it is important to be able to differentiate between these types of sounds. While being vigilant is adaptive in a new and/or hostile environment, being constantly alert and vigilant even if the environment is more favorable may be maladaptive. The finding that individuals exposed to early life stress are more vigilant (which is a key characteristic of anxious individuals) is consistently found in animal studies (Gunnar & Quevedo, 2007; Lutz & Turecki, 2014; Weinstock, 2005, 2008), while the assocation between maternal anxiety during pregnancy and childhood anxiety or emotional problems is found in some human studies (O'Donnell, Glover, Holbrook, & O'Connor, 2014; O'Connor, Heron, Golding, & Glover, 2003; Van den Bergh & Marcoen, 2004). Thus, we looked for early markers of these traits or problems in infants exposed to varying levels of maternal anxiety during prenatal life. Moreover, as human DOBHaD studies have almost exclusively focused on the effects of negative maternal emotions during pregnancy on child neurocognitive function, the focus of this study was expanded to include exposure to positive maternal emotions to determine whether and how they might also influence fetal (brain) development. Such a focus was both theoretically interesting and clinically relevant. For instance, Lobel, DeVincent, Kaminer, and Meyer (2010) had shown that in women with high-risk pregnancies, optimism was a key protective factor against adverse pregnancy outcomes. Thus, a good candidate for our study was a positive trait, such as mindfulness (Keng, Smoski, & Robins, 2011). Being mindful refers to a state of mind consisting of two key elements: (1) An alert mode of perceiving all mental contents (i.e., perceptions, sensations, cognitions, and emotions) and (2) a friendly, accepting, and nonjudgmental attitude towards those mental contents (Kohls, Sauer, & Walach, 2009). During pregnancy, experiencing positive emotions due to these two factors may enhance a pregnant woman's resilience against stress and adversity occuring during the pregnancy and hence constitute a prenatal environment that positively influences fetal brain development.

To study the relationship between maternal anxiety and mindfulness and offspring outcome, data from 79 Dutch mother–infant pairs were employed (van den Heuvel et al., 2014). At 20 weeks of gestation, women reported anxiety using the Symptom Checklist (SCL-90; Arrindell & Ettema, 1981, 2003) and mindfulness using the Freiburg Mindfulness Inventory (FMIs-14, Walach, Buchheld, Buttenmuller, Kleinknecht, & Schmidt, 2006). When their infants were 9 months-of age, EEG and auditory elicited ERPs were recorded using a passive auditory oddball paradigm. The stimulus sequences consisted of four different types of 200 ms sound events with an interstimulus interval of 300 ms, namely the standard sound and three deviant sounds (i.e., a white noise segment, a unique environmental sound such as slamming a door, and the same sound as the standard sound but with an interval of 100 ms). The frequent standard had a probability of 0.70 and the three types of deviants each had a probability of 0.10. A total of 1500 stimuli were delivered.

Mixed-mode ANOVAs were employed in two separate analyses, including the infants' mean ERP amplitudes (elicited by each of the four types of sound events) and either maternal anxiety or maternal mindfulness as a predictor. Preliminary analyses showed no effects of gestational age, birth weight or maternal anxiety at 9 months after delivery and they were not included in subsequent modelling. The results showed that higher maternal mindfulness (during the second trimester) was associated with smaller infant N250 and higher infant P150 ERP amplitudes to the standard sound while higher maternal anxiety (during the second trimester) was associated with larger N250 amplitudes to the standard sound. No effects were found for the three deviant sound stimuli.

From these results, we concluded that infants prenatally exposed to higher levels of maternal mindfulness devote less in-depth processing to repeated sounds with low information content, suggesting fast habituation to these sounds. In contrast, infants prenatally exposed to higher levels of maternal anxiety processed such uninformative sounds more extensively and/or they habituated more slowly to these stimuli. We speculate that

the 9 month-old infant ERP directional differences observed here to higher maternal mindfulness and anxiety during pregnancy might stem from infants prenatally exposed to higher maternal mindfulness pre-attentively forming more accurate perceptual representations, as reflected in higher P150 amplitudes to the standard sound. If so, a mindfulness intervention for pregnant women suffering from anxiety may be a desirable alternative or adjunct to pharmacological interventions. Clearly, firm conclusions await future research (van den Heuvel et al., 2014). The findings in relation to higher maternal anxiety during pregnancy are consistent with the results of other studies, namely with those showing that children prenatally exposed to high maternal anxiety have poorer language acquisition (King & Laplante, 2005; Laplante et al., 2004; Laplante, Brunet, Schmitz, Ciampi, & King, 2008) and are more anxious (O'Connor et al., 2003; Van den Bergh & Marcoen, 2004).

The results of Study 3 can be integrated in the DOBHaD model in the following way: prenatal exposure to maternal anxiety (early life events) in interaction with supposed (epi)genetic factors lead to altered emotion and (neuro)cognition and may evolve into increased vigilance (constituting the programmed phenotype).

As well as investigating the effects of anxiety during pregnancy on later offspring development, we also examined the effects of resolved anxiety disorders during pregnancy on infant development.

Study 4: Heart Rate Variability in Pregnant Women and Their Infants

At the time that we began this research, from our own studies (see above) and that of others (e.g., Alder, Fink, Bitzer, Hösli, & Holzgreve, 2007; Ross & McLean, 2006), it was known that active anxiety disorders and experiencing a high anxiety level had long-term detrimental effects on pregnant mothers and their offspring. However, it was unknown if a resolved, nonactive, maternal anxiety disorder had similar effects. Anxiety-related conditions, such as reduced autonomic cardiac control, indicated by reduced heart rate variability (HRV) could persist despite disorder resolution, with long-term health implications for mothers and children (Braeken et al., 2013). The autonomic nervous system of the fetus seems to be susceptible to the influence of maternal cardiac characteristics (Young, 2002). Indeed, it has been shown that HRV of the developing fetus is altered in the offspring of mothers with a number of psychiatric conditions, including anxiety disorders, and these differences persist postnatally (Dierckx et al., 2009; DiPietro et al., 2000; Monk et al., 2004). Thus, we designed this study to test the hypothesis that pregnant mothers with a history of, but not current anxiety disorder, and their children have low HRV, predicting offspring anxiety-like temperament (Braeken, 2014; Braeken et al., 2013).

To test the hypothesis, a case-control study including 56, 1st trimester Dutch women ($n=22$ with a history of anxiety disorder; $n=34$ with no history of psychopathology determined using the Mini-International Neuropsychiatric Interview 6.0, Sheehan & Lecrubier, 2010) and their offsprings was carried out. Anxiety was measured with the State Trait Anxiety Inventory (Spielberger et al., 1970; Van der Ploeg & Defares, 1980) and maternal ECG (to obtain maternal HR and HRV) was measured continuously during rest and mental stress. Stress was induced during a mental task. Each mother participated in a 25-min task that consisted of five testing phases, lasting 5 min each (Vlemincx, Taelman, De Peuter, Van Diest, & Van Den Bergh, 2011). Stress was induced in the second and fourth phases, with the remainder being relaxation phases. The stress consisted of mentally solving a complex mathematical problem such as $(361+11)\div(3\times4)+137$ without verbalization and selecting the answer from three choices presented on a computer screen. The relaxation phases consisted of viewing pictures considered peaceful and listening to music considered restful. At 2–4 months of age, infant ECGs were recorded. At 9–10 months of age, infant fearfulness was assessed using the unpredictable mechanical toy paradigm of the fear subscale of the Laboratory Temperament Assessment Battery (Lab-TAB)-Locomotor Version (Goldsmith & Rothbart, 1999) (Braeken et al., 2013, p. 2–3).

Repeated measures ANOVA controlling for mother's age and prepregnancy BMI, showed that HRV was lower in women in the past anxiety group compared to controls on both the root mean square of successive differences (RMSSD) and the high frequency (HF) measures of HRV. Regression analysis indicated that there was a significant relationship between maternal HRV measures and child HRV measures only in the anxiety group. Simple effects analysis showed that children of mothers with a past anxiety disorder had lower HRV (for both RMSSD and HF measures) than those born to mothers without a past anxiety disorder history. For all children, low HRV measures at 2–4 months were associated with a higher chance of fearful behavior at 9–10 months.

These results revealed that pregnant women with a past anxiety disorder had autonomic alterations (reduced parasympathetic function, indexed by HRV) early in pregnancy which may have influenced a subsequent physiological (reduced parasympathetic function, indexed by HRV) and/or psychological (fearful temperament) attribute of their offspring. The findings were independent of variations in maternal state-anxiety, age, sex, or body mass index. Additionally, mother–child associations were not explained by the children's birth weight or gestational age. The mechanisms by which a previous maternal anxiety disorder and/or HRV become associated with parasympathetic nervous system function in the offspring are unknown and a matter of speculation. It could be that altered autonomic function in pregnant women modulates their fetus' development. There is some support for this postulate as reduced HRV has been shown to be associated with dysregulation of several allostatic systems, including glucose regulation, hypothalamic–pituitary–adrenal axis function and inflammatory processes (Thayer & Lane, 2007; Thayer & Sternberg, 2006; Thayer, Yamamoto, & Brosschot, 2010) all of which may modulate fetal development (Lupien et al., 2009; Matthews & Phillips, 2010; Meyer et al., 2006; Van den Bergh, 2011; Van den Bergh, Mennes, et al., 2005; Young, 2002). However, whether altered maternal ANS function is causative or simply the result of shared underlying processes is unknown. Alternatively, given that mothers and their children share genes and environmental exposures, maternal behavior also may be an important factor in the observed associations (Rutter, 2002; Stern, 2009; Weaver et al., 2004) as well as shared genes. For example, research has shown that the combination of a brain-derived neurotrophic factor (BDDNF) V/V genotype and early life stress predicts changes in brain structure that are associated with lower HRV and higher anxiety (Gatt et al., 2009). These findings may explain, in part, why the women with a history of an anxiety disorder in this study demonstrated lower HRV. Whether it may account for our observation of a relationship between maternal–infant HRV is unknown (Braeken et al., 2013, p. 6).

Our DOBHaD model (Fig. 14.1) shows the effects of prenatal exposure to resolved maternal anxiety and altered ANS function (early life events) leading to altered ANS function and fearful temperament, which may evolve into a pro-anxiety phenotype (constituting the programmed phenotype).

Conclusion: The Use and Strength of the DOBHaD Model and Clinical Implications

In the past 30 years and especially in the last decade, an increasing number of studies have provided continuing evidence for an association between prenatal exposure to maternal stress, anxiety and depression, and altered behavior in the offspring. This body of evidence indicates that it may indeed be the case that events prior to birth, such as maternal emotions during pregnancy, *influence the way offspring respond to their postnatal environment* as demonstrated by correlations with infant outcome in the studies described above. The child of a highly anxious pregnant mother, by adapting to the early exposures when he/she was a fetus, reacts differently than the child of a low anxious pregnant mother, as reflected in newborn activity and EEG-responses, HRV, and temperament in infancy (see above studies), a delay in language development in toddlers and an enhanced risk for behavioral and emotional problems in childhood, specific cognitive problems,

anxiety and depression in adolescence and young adulthood (for recent reviews see: Beydoun & Saftlas, 2008; Bock, Poeschel et al., 2014; Braeken, 2014; Charil, Laplante, Vaillancourt, & King, 2010; Glover, 2011, 2014, 2015; Glover, O'Connor, & O'Donnell, 2010; O'Donnell, O'Connor, & Glover, 2009; Graignic-Philippe, Dayan, Chokron, Jacquet, & Tordjman, 2014; Henrichs & Van den Bergh, 2015; Lewis, Galbally, Gannon, & Symeonides, 2014; Lewis & Olive, 2014; Loomans, 2013; Loomans et al., 2011, 2013; Mennes, 2008; O'Connor et al., 2014; Otte, 2013; Räikkönen, Seckl, Pesonen, Simons, Van den Bergh, 2011; Schlotz & Phillips, 2009; Van den Bergh & Henrichs, 2015; Van den Bergh et al., 2015; Van den Bergh, Mulder, et al., 2005; Weinstock, 2008).

To understand how exposure to prenatal (and early postnatal) environmental events may influence later behavior, health and disease, several models have been developed (see for example Bock, Rether et al., 2014; Nederhof & Schmidt, 2012). However, no firm conclusion can yet be drawn about the validity of the different models (Daskalakis et al., 2013). In recently generated models (Bock, Rether et al., 2014; Daskalakis et al., 2013; de Kloet et al., 2014; Hanson & Gluckman, 2014; Lewis et al., 2014), including our own (Van den Bergh, 2010, 2011a), early life events typically are seen as "*conditional determinants*" rather than as determinants which always/invariably lead to behavioral problems, disorder, or disease. Put simply, this means that an organism that was prenatally programmed (or organized) to be adapted to a particular environment, will gradually be behaving in an altered "biased" way. The "bias" constitutes his or her programmed phenotype and reflects the way the organism was adapted during its early development. Although early adversity will in some environments finally lead to disorder or disease, in other environments early adversity may constitute a possible source of adaptation (Daskalakis et al., 2013). For instance, according to the "mismatch hypothesis" only a mismatch between the early environment and later postnatal environment will lead to disorder and disease, while a match will not (Gluckman & Hanson, 2004; Hanson & Gluckman, 2014). Biological sensitivity (Boyce & Ellis, 2005) or differential susceptibility to the environment (Belsky & Pluess, 2009) models predict that some individuals are more susceptible than others to both the adverse and beneficial effects of, respectively, unsupportive and supportive environments. This genetic difference in sensitivity or susceptibility and the nature of the environment will influence how mental health or mental health problems are shaped; these processes covary with physical health and health problems.

The results of the four studies described above exemplified how "conditional determinants" (i.e., prenatal environmental events such as exposure to maternal anxiety, maternal mindfulness, a resolved maternal anxiety, and altered maternal ANS) might have influenced the course of fetal development, resulting in alterations in the function of the brain and motor systems as evidenced during fetal life and, gradually, in a "biased" response/altered phenotype to subsequent environmental inputs later in life. Using the results of Study 1 to illustrate, being a highly active fetus could be seen as an adaptation to the prenatal environment shaped by a highly anxious mother. The subsequent observation of an increase in neonatal activity might signify that the brain of the infants became shaped to facilitate a higher level of arousal during fetal life. It also could indicate an effect on self-regulation such that later in life, the infant, toddler and child would seek out arousal-inducing events. A consequence of arousal-seeking is that it could lead to hyperactivity and/or impulsivity, which places the child at risk for behavioral problems or attention deficit hyperactivity disorder (ADHD) in some, but not in other environments. For instance increased motor activity will in a (school) environment that requires restraint of impulsivity and motor activity be seen as inadequate behavior. However, in an environment that is stress-inducing and potentially harmful, or in a novel environment, increased motor activity (hyperactivity) may be adaptive (e.g., to explore the environment for threats and opportunities) (Jensen et al., 1997). In Study 3, infants of highly anxious pregnant women were more vigilant than those of low anxious pregnant women (i.e., ERPs indicated that they reacted stronger to a repeated, uninformative sound and seem to habituate less). In Study 4,

infants of pregnant women with a resolved anxiety disorder who had lower HRV measures of RMSSD and HF during pregnancy, also showed similar lower HRV measures at 2 months of age, which predicted an anxious-like temperament at 9 months of age. When these infants, with an altered phenotype [i.e., 'a bias in neurocognitive function (Study 3) and in sympathetic activity/fearfulness (Study 4)] encounter an anxiety or fear-inducing, environment such behavior may have an adaptive value. According to some authors (e.g., Hanson & Gluckman, 2014; Lee & Goto, 2013; Lewis et al., 2014; O'Connor et al., 2014) this bias, which also can be interpreted as a higher degree of stress reactivity, may in fact be promoted by maternal anxiety during pregnancy. The mother signals, with physiological changes accompanying anxiety, aspects of the environment to which the fetus adapts because it may have adaptive value if he or she encounters a similar (i.e., a matched) anxiety-inducing environment after birth/later in life (i.e., early life experience may program the brain for life to come). However if an individual is more vigilant in all types of environments (e.g., also in more favorable, safe ones), this biased (prenatally acquired) behavior may tax or compromise emotional and/or neurocognitive functioning and anxiety symptoms and poorer language acquisition may result. In order to develop adequate self-regulation skills, it will be vital that parents (and other educators) understand this fearfulness and/or heightened stress reactivity and try to induce changes in this behavior. Neural circuitry, molecular profiles, and neurochemistry can be (positively) changed by experiences; these changes will in turn influence subsequent behavior (Bai & Repetti, 2015; McEwen & Morrison, 2013). If therapeutic interventions are needed, they might target the underlying mechanisms that produce heightened stress reactivity as well as strategies that might prevent subsequent related behavioral problems or psychopathology (Schechter, 2012). Importantly, the fact that phenotypes are programmed by adaptation to early life environments does not imply that they cannot be changed! To refer to the amazing plasticity of the developing brain, Seymour Levine once said "Nothing is written in stone" (Levine, 2005); this statement has frequently been cited in early life stress literature (e.g., Daskalakis et al., 2013).

A strength of the studies used to generate our DOBHaD model, including the ones detailed above, is that they focused on processes (constituting elements) of behavior that could be measured in an objective, standardized way (e.g., changes in fetal behavioral state; event related potentials during sensory stimulation; heart rate variability measures). The importance of the findings and the model based on this line of research are their potential for identifying those maternal–offspring factors that could serve as markers of later mental health issues. A recent Danish population based study indicates that a person exposed to prenatal stress makes more use of primary health care than a person not exposed to prenatal stress (Li, Yang, Guldin, Vested, & Vestergaard, 2015). However, only when markers and underlying mechanisms are identified, may interventions be developed that are targeted to prevention and/or amelioration of specific health issues. Such interventions may focus on the care given to the mother during pregnancy, the mother herself, or the offspring. Recent randomized controlled trial studies of interventions to treat anxiety and depression during pregnancy show not only improvements in maternal mental health but in infant outcome (e.g., better self-regulation and stress reactivity) as well (e.g., Milgrom et al., 2015).

The human and economic toll of mental health issues in the population is substantive. A recent UK report (Bauer, Parsonage, Knapp, Iemmi, & Adelaja, 2014) calculated the costs of mental health problems (i.e., depression, anxiety, psychosis, post-traumatic stress disorder) during pregnancy and the first year after childbirth at about £8.1 billion for each 1-year cohort of births and the equivalent of just under £10,000 for a single birth. About 72 % of this cost relates to adverse effects on the child rather than the mother. It seems that, not only for fundamental scientific studies, but also for clinical studies and for society as a whole, the study of prenatal environmental influences on offspring outcome is critical. It has considerable potential for improving behavior and health outcomes because maternal anxiety, stress and lifestyle are modifiable.

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Part IV

Environmental Influences on Development: Iron Deficiency, Alcohol Consumption, SSRI Exposure

Long-Term Brain and Behavioral Consequences of Early-Life Iron Deficiency

15

Bruce C. Kennedy, Diana J. Wallin, Phu V. Tran, and Michael K. Georgieff

Abstract

Early-life iron deficiency anemia affects 30–50 % of pregnancies worldwide and causes deficits in cognitive development as well as socio-emotional abnormalities. More concerning, these deficits persist into adulthood, including increased risks of schizophrenia and depression, despite prompt iron repletion during childhood. Emerging evidence implicates long-term changes in the neural metabolome, proteome, and genome as potential biological bases underlying these effects. In turn, better knowledge of the underlying biology will lead to new methods of identifying young children at risk for brain iron deficiency and adjunct or rescue therapies designed to optimize their outcomes.

Keywords

Iron • Fetus • Infant • Dopamine • Epigenetics • Cognition • Genomics • Proteomics • Developmental origins

Introduction

Iron deficiency is the most prevalent nutrient deficiency in the world. The World Health Organization (WHO) estimates that 30–50 % of pregnant women and preschool age children worldwide are iron deficient (ID) anemic (McLean, Cogswell, Egli, Wojdyla, & de Benoist, 2009). The majority of children at risk for iron deficiency are either newborns or infants between the ages of 6 months and 3 years. Common maternal-gestational conditions that increase the risk for late gestational and neonatal (perinatal) iron deficiency anemia include severe maternal

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iron deficiency anemia, hypertension-induced placental vascular insufficiency, diabetes mellitus, and cigarette smoking during pregnancy (Chockalingam, Murphy, Ophoven, Weisdorf, & Georgieff, 1987; Petry et al., 1992; Sweet, Savage, Tubman, Lappin, & Halliday, 2001). The most common postnatal cause of iron deficiency anemia is inadequate dietary iron intake often combined with intestinal blood loss. Importantly, both time periods exhibit the principle that early-life iron deficiency causes long-term neurobehavioral deficits (Lozoff, 2011; Lozoff et al., 2006). Using animal models, investigations into the molecular underpinnings of the behavioral effects induced by early-life iron deficiency reveal acute and long-term abnormalities in multiple aspects of the brain (Georgieff, 2011).

Historical perspective of research on brain effects induced by early-life iron deficiency anemia

As early as 1959, the WHO identified iron deficiency anemia as a major public health concern, in particular for vulnerable populations (e.g., pregnant women, infants, and young children) ("Iron deficiency anaemia; report of a study group," 1959). In the following decades, iron deficiency anemia was recognized as one of the most common nutrient deficiencies worldwide (Baker & DeMaeyer, 1979). Although an estimate of the affected population is a subject of debate, a conservative estimate suggests two billion individuals were ID worldwide during the period of 1985–2000 and the prevalence continues to climb (Stoltzfus, 2001). The first experimental models of early postnatal iron deficiency postulated that iron deficiency impairs energy metabolism and thereby restricts tissue growth and development (Dallman, 1969; Salmon, 1962). Following up along this line of investigation, Georgieff and colleagues later (1980s–1990s) identified populations at risk for fetal and newborn brain iron deficiency (Chockalingam et al., 1987; Georgieff, Berry, Wobken, & Leibold, 1999; Petry et al., 1992) and established that fetal–neonatal iron deficiency affects the development of the hippocampus, a brain region critical for learning and memory (Fretham, Carlson, & Georgieff, 2011). Early iron deficiency also affects other brain regions during development (Beard, Erikson, & Jones, 2003; Wu et al., 2008). In the late 1970s, Youdim and colleagues found that because iron is required for the synthesis of monoamine neurotransmitters, early iron deficiency alters the production of brain dopamine and noradrenaline in young rats (Ben-Shachar, Ashkenazi, & Youdim, 1986; Youdim & Green, 1978). Subsequently, Jones and colleagues confirmed these findings and found changes to serotonin, particularly in limbic regions (e.g., prefrontal cortex, hippocampus, and nucleus accumbens) (Beard et al., 2003). These findings were postulated to underlie iron deficiency-induced dopaminergic-driven neurobehavioral dysfunction (Youdim, Ashkenazi, Ben-Shachar, & Yehuda, 1984; Youdim, Yehuda, & Ben-Uriah, 1981). Leveraging this information in studies that began in the early 1980s, Lozoff and colleagues have observed cognitive and behavioral abnormalities consistent with alterations in dopaminergic metabolism in iron-deficient anemic human infants (Lozoff et al., 2006; Lozoff, Wolf, Urrutia, & Viteri, 1985). More concerning is the observation that the cognitive and socio-emotional deficits noted while ID persist into adulthood despite prompt treatment of iron deficiency (Lozoff, 2011). These observed behavioral abnormalities in humans also have been demonstrated in animal models of early-life iron deficiency (Ben-Shachar et al., 1986; Lubach & Coe, 2008). The effects of iron deficiency on fatty acid metabolism also have been shown in rodent models since the 1990s (Connor & Menzies, 1996). Subsequent studies in human infants that show slower nerve conduction speed seem to corroborate this effect (Amin et al., 2010; Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998). The question as to whether the effects seen in humans and preclinical models are truly due to loss of iron (as opposed to the effects of anemia) was answered by recent studies using transgenic mouse models of neuronal iron deficiency without anemia. These studies established that the lack of neuronal iron is key to

the observed neurocognitive learning and memory deficits of early-life iron deficiency anemia (Carlson et al., 2009; Fretham et al., 2012). The most recent work in the field has concentrated on the long-term effects of early-life iron deficiency. While it is not surprising that an ID brain functions poorly, the causes behind the persistence of deficits into adulthood in both humans and preclinical models remains an interesting and unsolved problem. The discovery that early-life iron deficiency alters brain gene expression during the period of deficiency and long after repletion may provide some clues to underlying mechanisms.

Behavioral Effects of Early-Life Iron Deficiency Anemia

Early-life iron deficiency anemia impairs cognitive and socio-emotional functions

Acute effects during early-life iron deficiency anemia

Early-life iron deficiency anemia impairs cognitive and socio-emotional behavior development. The acute effects of early-life iron deficiency have been known for many years. Early studies on cohorts of infants with iron deficiency anemia in Central and South America revealed that moderate to severe iron deficiency anemia results in acute impairments on the Mental Development Index (MDI) in the Bayley's Scales of Infant and Toddler Development (BSID) (Lozoff et al., 1987; Lozoff, Brittenham, Viteri, Wolf, & Urrutia, 1982), a measure which strongly correlates with childhood IQ. Within the domain of cognitive performance, ID infants have specific deficits in recognition memory (Carter et al., 2010; Siddappa et al., 2004), object permanence (Carter et al., 2010), reversal learning (Soewondo, Husaini, & Pollitt, 1989), and attention (Lozoff et al., 1998).

ID infants also are more likely to score lower on measures of socio-emotional development (Lozoff et al., 1987). ID infants show more wariness and hesitancy than iron sufficient (IS) infants, and are less likely to engage examiners (Lozoff et al., 1998). For example, although free-play behavior is normal, ID infants are slower to approach examiners during play solicitation (Lozoff et al., 2008) and faster to return to their mothers (Lozoff et al., 2007). The reduced sociability of ID infants also is evident in higher maternal ratings of shyness (Lozoff et al., 2008) and decreased social referencing (Lozoff et al., 2003, 2007). Furthermore, ID infants exhibit blunted positive affect (Lozoff et al., 1998) and increased fearfulness toward unfamiliar people and objects (Carter et al., 2010; Lozoff et al., 1998). Finally, ID infants tend to exhibit language deficits (Walter, De Andraca, Chadud, & Perales, 1989) and are less likely to vocalize to solicit interaction than their IS counterparts (Lozoff et al., 1998). Overall, the socio-emotional deficits of ID infants can be characterized as increased withdrawal from novel stimuli as well as a reduced tendency to initiate social interactions using language or approach, which may be related to the blunted affect and social motivation of ID infants.

These findings lead to the notion that the neurocognitive impairments of ID infants may be caused or exacerbated by the socio-emotional deficits. In support of this proposal, measures of orientation/engagement on the Behavioral Rating Scale were predictive of performance on an object permanence task in ID infants (Carter et al., 2010) and MDI scores were lower in ID infants who also exhibited abnormal affect (Lozoff et al., 1985). These findings suggest that the reduced ability of ID infants to engage their environment and solicit attention from caregivers may further hamper cognitive and socio-emotional development, leading to a cycle of underperformance. This prediction, referred to as the functional isolation hypothesis (Levitsky & Barnes, 1972; Levitsky & Strupp, 1995), is an important consideration when evaluating different treatment options. If cognitive impairments are exacerbated by the poor socio-emotional behaviors, programs aimed at improving social interactions with caregivers may be a necessary component of any successful treatment approach.

Chronic effects beyond the period of iron deficiency anemia

Due to slower growth rates and more substantial iron content in post-weaning diets, iron status tends to improve as ID children reach school age (Zimmermann & Hurrell, 2007). However, despite normalization of iron status, the period of iron deficiency anemia during fetal and perinatal development results in long-term consequences on behavior. Presently, ID infants from early cohorts in Chile and Costa Rica have received follow-up evaluations up to 10 (Lozoff, Castillo, Clark, Smith, & Sturza, 2014) and 25 years (Lozoff et al., 2013), respectively, after the initial study. Compared to relatively limited tests available to assess neurobehavioral outcomes of infants less than 1 year old, researchers have utilized a more substantial set of tools for evaluating behavioral deficits in formerly iron deficient (FID) children, adolescents, and adults.

Generally, the cognitive deficits of FID children and young adults as measured by global indices are similar in magnitude to those observed in ID infants. A longitudinal study that followed infants into childhood and adolescence found that the discrepancy between the cognitive scores of ID and IS infants was maintained up to 19 years of age if the infants came from families of middle socioeconomic status (SES) and this gap increases over time if ID infants came from low SES families (Lozoff et al., 2006). However, compared to studies of ID infants there is much more detailed information about the specific functions that are impaired in FID individuals relative to an always IS comparison group. At 5 years of age, FID children exhibited slower perceptual speed and poorer understanding of quantitative concepts (Lozoff, Jimenez, & Wolf, 1991) as well as impaired language abilities (Tamura et al., 2002). In early adolescence, FID individuals were characterized as having slower perceptual speed, poorer spatial memory, and developmental delays in a selective attention task (Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000). These impairments may contribute to the lower scores of FID adolescents on tests of reading and arithmetic as well as the increased likelihood to repeat a grade or receive referrals for tutoring (Lozoff et al., 2000). As young adults, FID individuals performed more poorly on measures of recognition memory and strategy shifting (Lukowski et al., 2010). Finally, outcome measures of employment and education at 25 years suggest that the cognitive deficits experienced throughout infancy, childhood, and adolescence had a significant impact on the lives of FID adults. A larger proportion of FID adults did not complete secondary school and were not actively pursuing further training or education (Lozoff et al., 2013).

Similar to cognitive performance, there are parallels between the socio-emotional deficits of ID infants and FID children and adults. At ages 4–5, FID children exhibited reduced positive affect during an interactive task with their mother (Corapci, Radan, & Lozoff, 2006) or a stranger (Chang et al., 2011) and were found to be more passive and unengaged than always IS children (Chang et al., 2011; Lozoff et al., 2007). FID children also displayed poorer self-control on a delayed-gratification task (Chang et al., 2011). As early adolescents, FID individuals received higher ratings by parents or teachers on measures of anxiety and depression, social problems, delinquent behavior, and aggression (Lozoff et al., 2000). Finally, FID adults self-reported more feelings of detachment and negative emotions and had lower ratings for emotional health (Lozoff et al., 2013).

Considered as a whole, the longitudinal behavior data from ID infants and FID children, adolescents, and adults seems to point to a specific set of cognitive and socio-emotional deficits following early-life iron deficiency. Within the cognitive domain, early-life iron deficiency leads to long-term impairments in perceptual speed and attention, particularly when a task requires selective attention toward task-relevant (versus task-irrelevant) information. A distinct but perhaps related deficit can be found for hippocampal-dependent memories such as spatial and recognition memory. From a socio-emotional perspective, early-life ID and FID individuals appear to experience fewer positive emotions during activities or social encounters that are normally pleasurable. FID individuals are also socially with-

drawn and prone to anxiety, particularly toward unfamiliar people or things.

These longitudinal studies have provided additional evidence supporting the functional isolation hypothesis through evaluations of reciprocal interactions between FID children and caregivers. Corapci and colleagues (2006) measured developmentally facilitative mother–child interactions such as shared affect, eye contact, and turn taking and found that mother–child reciprocity was lower among pairs with FID children. Furthermore, the mothers of FID children also were found to be less responsive toward their children. These findings may suggest that the withdrawn nature of ID infants and FID children influences the behavior of caregivers, making them less likely to attempt to engage the child in interactions that may be important for normal social development.

Early-life iron deficiency anemia impairs motor function and alters sleep–wake cycles

Some of the most widely known consequences of iron deficiency anemia are lethargy and fatigue. However, reduced activity level is just one long-term effect of iron deficiency anemia on motor behavior, arousal and sleep–wake cycles which can include increased susceptibility to attention deficit hyperactivity disorder (Konofal, Lecendreux, Arnulf, & Mouren, 2004) and restless leg syndrome (Peirano et al., 2012) later in life. Although an in-depth review of the link between iron deficiency and these disorders is outside the scope of this chapter, the subject has been previously reviewed (Allen, 2004; Cortese et al., 2008).

Acute effects during early-life iron deficiency anemia

ID infants with or without anemia tend to be delayed on developmental motor milestones (Shafir et al., 2008) and score lower than IS individuals on global indices of motor function and coordination (Lozoff et al., 2007). Specifically, researchers have noted that ID infants have difficulty with normal balance and weight bearing while standing at 1 year of age (Lozoff et al., 1987; Shafir et al., 2008; Walter et al., 1989). Furthermore, ID infants tend to be generally less active, exhibiting lower levels of spontaneous activity (Angulo-Kinzler, Peirano, Lin, Garrido, & Lozoff, 2002; Olney et al., 2007). Activity data across sleep–wake cycles of ID infants also revealed increased likelihood of fragmented sleep and abnormal EEG patterns relative to IS individuals (Angulo-Kinzler et al., 2002; Peirano, Algarín, Garrido, Algarín, & Lozoff, 2007).

Chronic effects beyond the period of iron deficiency anemia

Despite the resolution of iron deficiency, FID children were more likely to score below average on scales of fine motor abilities at 5 years (Tamura et al., 2002) and general motor function at 10 years (Lozoff et al., 2000). Similarly, even at 4 years of age, FID children show evidence of an abnormal sleep rhythm, including more activity during sleep, and reduced sleep during daytime hours (Peirano et al., 2012; Peirano, Algarín, Garrido, & Lozoff, 2007).

Preclinical Models of Early-Life Iron Deficiency Anemia

In addition to studying ID infants, researchers have characterized the effects of early-life iron deficiency in preclinical models. In contrast to human cohorts where the effects of iron deficiency may be confounded by other nutritional deficiencies or overlooked environmental factors, rodent models provide an opportunity to directly determine how iron deficiency alters cognitive and socio-emotional behavior. Furthermore, the use of transgenic mice allows researchers to induce iron deficiency in specific brain structures (Carlson et al., 2009) or at specific stages of development (Fretham et al., 2012). Finally, rodent and other preclinical models are ideal for preclinical assessment of potential preventative measures or treatments to reverse the effects of fetal–neonatal iron deficiency. However, studies using rodent models vary considerably in terms of the timing and severity of the iron deficiency. Although this may compli-

cate direct comparison of findings from different studies, these discrepancies have helped to uncover factors that are the most important predictors of long-term behavioral outcomes.

Early-life iron deficiency anemia results in cognitive and socio-emotional deficits in preclinical models

Acute and chronic effects of early-life iron deficiency anemia

Despite the numerous studies documenting the effects of acute iron deficiency on measures of cognitive and socio-emotional performance in human infants, relatively few studies have explored the acute effects of iron deficiency in infant rodents. Eseh and colleagues took advantage of the tendency for infant rat pups to vocalize when separated from the dam in order to determine whether maternal separation is more stressful to ID pups. They found that ID pups produced more distress vocalizations following separation (Eseh & Zimmerberg, 2005), which appears consistent with the tendency of ID infants to remain within close proximity to caregivers and to be wary of strangers. In addition, rats in early adolescence exhibit deficits in hippocampal-dependent trace fear conditioning (Gewirtz, Hamilton, Babu, Wobken, & Georgieff, 2008). Although brain iron measurements were not taken in these animals, a similar gestational model of iron deficiency resulted in a reduced brain iron concentration as late as postnatal day (P)28 despite a return to IS diet at P7 (Rao, Tkac, Townsend, Gruetter, & Georgieff, 2003), which would suggest that rats used in the Gewirtz et al. study were still brain ID.

Consistent with the findings of the longitudinal human studies described above, early-life iron deficiency results in long-term cognitive and socio-emotional consequences in rodent models. Furthermore, the deficits observed in FID rodents tend to mirror those in FID children and adults. For example, FID rats exhibit impairments on hippocampal-dependent memory tasks, including both spatial (Bourque, Iqbal, Reynolds, Adams, & Nakatsu, 2008; Carlson et al., 2009; Felt et al., 2006; Schmidt, Waldow, Grove, Salinas, & Georgieff, 2007) and recognition (Kennedy et al., 2014) memories as well as trace fear conditioning (McEchron, Cheng, Liu, Connor, & Gilmartin, 2005) and passive avoidance (Harvey & Boksa, 2014). The behavioral phenotype of FID rats is also consistent with the heightened wariness and neophobia of FID infants, although results have been mixed. Some studies have reported normal anxiety-like behavior in FID animals (Eseh & Zimmerberg, 2005; Felt & Lozoff, 1996), while other groups have consistently found abnormalities in FID rats on more subtle measures of anxiety such as increased latency to explore novel or open areas (Bourque et al., 2008; Felt et al., 2006), reduced approach toward unfamiliar animals (Harvey & Boksa, 2014; Kennedy et al., 2014), or slower habituation to novel surroundings (Eseh & Zimmerberg, 2005).

Early-life iron deficiency anemia results in motor function and activity deficits in preclinical models

Acute and chronic effects of early-life iron deficiency anemia

Consistent with human studies, early-life iron deficiency impairs the motor development of young rodents. For example, at multiple time points from P6–16, ID rats exhibit deficits in neurological reflexes with a motor and coordination component, such as the righting and negative geotaxis reflex (Unger et al., 2012; Wu et al., 2008). Likewise, ID rats display less exploratory locomotion as pups and adolescents (Eseh & Zimmerberg, 2005; Piñero, Jones, & Beard, 2001), a potential behavioral correlate of spontaneous activity measured in human infants and children.

Long-term effects of early-life iron deficiency on motor function have been mixed, with support for both continued motor deficits (Felt et al., 2006; Ward et al., 2007) and recovered motor function (Harvey & Boksa, 2014; Kwik-Uribe, Golub, & Keen, 2000) following iron repletion. However, the type of motor behaviors measured

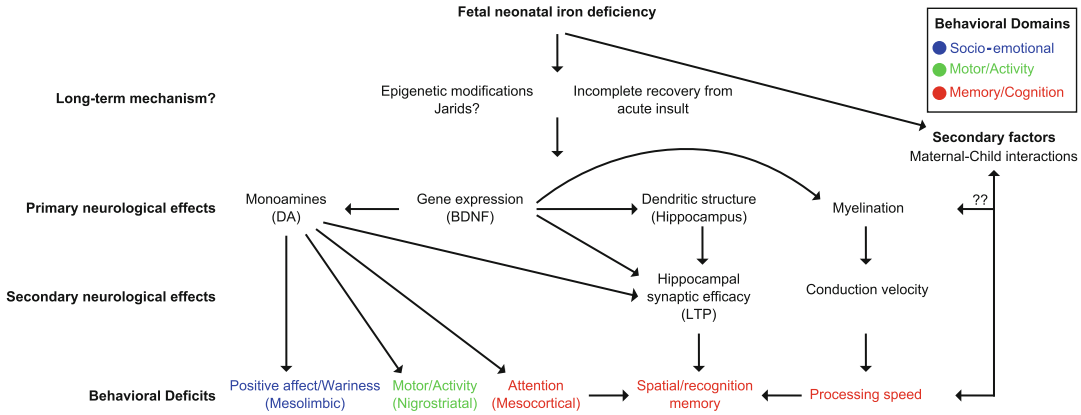

Fig. 15.1 Putative neurobiological mechanisms underlying the long-term behavioral effects of early-life iron deficiency. Early-life iron deficiency results in long-term neurobiological and behavioral deficits despite resolution of brain iron deficiency prior to adulthood. These long-term outcomes are hypothesized to occur either through stable epigenetic modification of genes or through incomplete recovery from acute iron deficiency experienced during fetal and neonatal development. The primary neurological effects observed following early-life iron deficiency include decreased monoamine neurotransmission, reduced expression of neurotrophic factors such as BDNF, altered dendritic structure in the hippocampus, and decreased myelination. These primary effects are proposed to lead to systems-level deficits in conduction velocity and hippocampal synaptic efficacy, measured via LTP, and likely underlie the long-term changes in behavior. Alterations in monoamine dopamine (DA), in particular have been linked to specific behavioral changes consistent with the major dopaminergic pathways. In addition to direct effects, early-life iron deficiency is known to alter mother–infant interactions which likely has a bidirectional relationship with long-term behavioral outcomes

varied amongst studies and thus discrepancies may reflect differential effects of early-life iron deficiency on different aspects of motor function, such as coordination, speed, strength, or balance. Similarly, there is evidence for a lasting decrease in exploratory activity in FID adult rats (Beard et al., 2003; Bourque et al., 2008), while other groups have reported that these behaviors had normalized (Eseh & Zimmerberg, 2005; Felt et al., 2006; McEchron et al., 2005). Persistent reductions in activity levels also were found in FID rhesus monkeys, although fine and gross motor function was normal in these animals (Golub et al., 2006).

Potential Mechanisms Underlying Acute and Long-Term Behavioral Deficits of Early-Life Iron Deficiency Anemia

Although the exact relationships between biochemical or physiological changes resulting from early-life iron deficiency and specific functional outcomes are still relatively unknown, it is clear that taken as a whole, the cognitive, socio-emotional, and motor deficits observed in FID individuals closely align with the neurobiological substrates that suffer long-term effects (Fig. 15.1). By establishing that the acute and long-term behavioral deficits are consistent with the most prominent and well-established changes in neural systems or processes, the iron deficiency field is well poised to further map out the causal mechanisms and design treatments for the impairments resulting from early-life iron deficiency.

Early-life iron deficiency alters brain dopaminergic signaling

Ever since the demonstration that the production of neurotransmitters (i.e., dopamine and noradrenaline) is dependent on iron-containing tyrosine hydroxylase, it was speculated that iron deficiency affects neurotransmitter synthesis and metabolism. Indeed, Youdim and Green (1978)

confirmed this hypothesis using a young rat model of iron deficiency in their early work. Subsequent work from others has explored the link between iron deficiency and dopamine, both in the short- and long-term.

Acute effects during iron deficiency anemia

One of the first results connecting iron deficiency with dopamine was that of Youdim and collaborators, who found that postnatal iron deficiency resulted in a decrease in dopamine D2 receptors within the caudate-putamen (Youdim & Green, 1978). However, they did not find any alterations to dopamine concentrations or turnover. This prompted Beard and colleagues to use *in-vivo* microdialysis and they found an increase in steady-state extracellular dopamine concentration in caudate-putamen (Beard, Chen, Connor, & Jones, 1994). Following up on this work, Erikson and colleagues demonstrated a decrease in the density of dopamine transporter (DAT) as well as dopamine uptake in ID rodent striatum (Erikson, Jones, & Beard, 2000). Early-life iron deficiency anemia also results in a decrease in D1 and D2 receptor density within the striatum and D2 receptor density within the nucleus accumbens (Erikson, Jones, Hess, Zhang, & Beard, 2001). This decrease in receptor density within the striatum correlates with the loss of iron in the striatum, but not in other brain regions.

Behavioral measures have been correlated with these cellular findings. After 6 weeks of either ID or IS diet, weanling rats fed an ID diet showed a decrease in activity and an increase in anxiety-like behaviors. Ventral midbrain iron concentration was correlated with exploration while D1 receptor density was highly correlated with repeated movements. The anxiety-like behaviors correlated with prefrontal cortex DAT and D1 receptor density (Beard, Erikson, & Jones, 2002). In the prefrontal cortex, reduced concentrations of extracellular dopamine were associated with increased anxiety behaviors on the elevated plus maze task and decreased motor coordination as assessed by the accelerating rotarod (Li et al., 2011).

Chronic effects beyond the period of iron deficiency anemia

Importantly, DAT, D1, and D2 receptor levels do not normalize in the striatum with iron repletion following the early postnatal period of iron deficiency (Beard et al., 2003). Early-life iron deficiency can result in long-term brain abnormalities that manifest as behavioral deficits. In 2006, Felt et al. assessed the long-term consequences of early-life iron deficiency anemia on behavior, brain iron, and monoamine metabolism. Rats in these studies were given an IS or a low-iron diet throughout gestation and lactation. After weaning, all rats received an IS diet and hematology and growth in all animals was normalized. However, rats receiving a low-iron diet during early-life had persistent sensorimotor deficits including delayed vibrissae-evoked forelimb placing, increased hesitancy when placed in novel settings, and poorer performance on the Morris Water Maze, a test of spatial memory (Felt et al., 2006). These results suggest a persistent effect of iron deficiency in early life on the striatal dopaminergic system as well as the hippocampus. To specifically test the long-term effects on the dopaminergic pathway in humans, follow-up assessments were done comparing subjects who had chronic, severe iron deficiency as children to those with good iron status as children. Despite being fully IS during the neurocognitive testing, subjects who were formerly ID as children showed deficits in documented frontal-striatal-dependent set-shifting tasks, implicating dopamine dysfunction (Lukowski et al., 2010).

Early-life iron deficiency anemia alters neuronal myelination

Based on the fact that iron is required for the enzymatic activity of desaturases critical for the fatty acid profile of myelin, the effects of early-life iron deficiency anemia on brain myelin content have been investigated. One way to assess neuronal myelination is to quantify expression of myelin related proteins, including myelin basic

protein (Mbp). Mbp is a complex gene with multiple splice variants, encoding a major component of the myelin sheath of oligodendrocytes. Iron deficiency alters expression of Mbp and reduces myelination in preclinical models. These changes are likely correlated with the reduced neuronal conduction velocity seen in ID and FID humans, which may underlie the reduced speed of information processing (see previous section). For example, ID preterm neonates (Amin et al., 2010) and ID 6-month-old infants (Roncagliolo et al., 1998) exhibit longer latencies on auditory brainstem evoked responses. The persistence of prolonged latencies on visual evoked responses in FID 4-year-olds that have recovered from iron deficiency appears to confirm these effects are long lasting in humans (Algarín, Peirano, Garrido, Pizarro, & Lozoff, 2003).

Acute effects during iron deficiency anemia

Myelination is dependent on brain iron availability both directly through the requirement of iron for lipid synthesis as well as indirectly due to the high metabolic demand of myelin-producing oligodendrocytes (Connor & Menzies, 1996). As such, it is thought that iron is critical for the normal maturation of oligodendrocytes and growth of white matter tracts. Indeed, gestational iron deficiency drastically alters the number of oligodendrocytes in the developing CNS in a tissue-specific manner (Morath & Mayer-Pröschel, 2002). With regard to myelination, brain iron availability may be particularly important during early postnatal development during which a period of rapid myelination coincides with the peak of iron uptake into the brain (Taylor & Morgan, 1990). During this metabolically active developmental phase, acute perinatal iron deficiency resulted in delayed or reduced myelination in multiple areas within the P11 and P17 brain and spinal cord (Yu, Steinkirchner, Rao, & Larkin, 1986). In addition to morphological changes, acute perinatal and postnatal iron deficiency both resulted in reduced expression of oligodendrocyte proteins important for myelination, Mbp and 2′,3′-Cyclic nucleotide 3′-phosphohydrolase (CNPase) (Beard et al., 2003; Wu et al., 2008). Fetal–neonatal ID rats also showed an altered hippocampal neurochemical profile and decreased Mbp mRNA expression consistent with reduced myelination (Carlson, Stead, Neal, Petryk, & Georgieff, 2007; Rao et al., 2003). Similar to ID human infants, neuronal conduction velocity is altered in ID pups and adolescent rodents (Jougleux, Rioux, Church, Fiset, & Surette, 2014; Lee, Strathmann, Gelein, Walton, & Mayer-Pröschel, 2012) but was not necessarily associated with changes in myelin (Lee et al., 2012).

Chronic effects beyond the period of iron deficiency anemia

Altered levels of myelin components including lipids (Ortiz et al., 2004) and expression of myelin-associated genes also have been observed in the FID adult animal. For example, reduced expression of Mbp protein was observed in FID rats (Ortiz et al., 2004) and nonhuman primates (Patton, Coe, Lubach, & Connor, 2012). Similar findings of reduced expression of Mbp mRNA in the brain of FID rats (Clardy et al., 2006; Kennedy et al., 2014; Tran, Fretham, Carlson, & Georgieff, 2009) suggest that this decrease likely stems from permanent changes in gene transcription. Mechanistically, reduction of Mbp expression in FID rats may result from reduced Egr-2 expression, which regulates insulin-like growth factor 2 (IGF-II) and Mbp in astroglia (Gillian & Svaren, 2004; Jang, LeBlanc, Roopra, Wrabetz, & Svaren, 2006; Rotwein, Burgess, Milbrandt, & Krause, 1988; Ye, Li, Richards, DiAugustine, & D'Ercole, 2002). Finally, early ID-mediated changes in myelination seen in multiple studies suggest that metabolism may be the key to normal myelination during brain development (Todorich, Pasquini, Garcia, Paez, & Connor, 2009).

Early-life iron deficiency anemia alters hippocampal development and function

The hippocampus is a major component of neural networks that contribute to learning and memory. The hippocampus develops rapidly and has high metabolic demands from late fetal life through early childhood and thus serves as an

excellent brain structure to investigate the neural basis underlying the abnormal cognitive and affective behaviors caused by fetal–neonatal iron deficiency. While much of the research in preclinical models has concentrated on the specific effects of iron deficiency during late gestation on the neonatal hippocampus as a way of explicating learning and memory deficits in ID human newborns, the principles demonstrating the requirement for iron in rapidly differentiating neurons likely apply to other brain regions affected by early-life iron deficiency. These areas include the striatum, prefrontal cortex, and cerebellum.

Acute effects during iron deficiency anemia

Differentiation of hippocampal neurons occurs rapidly between P15 and P30 in rats (Pokorný & Yamamoto, 1981; Steward & Falk, 1991) with a corresponding increase in iron uptake (Siddappa et al., 2002) and expression of genes associated with neural differentiation and plasticity (Tran, Carlson, Fretham, & Georgieff, 2008). A summary of critical events for hippocampal development during late gestational and early postnatal life is reviewed elsewhere (Fretham, Carlson, & Georgieff, 2011). These findings demonstrate the vulnerability of the hippocampus to disturbances in metabolites (e.g., oxygen, iron, glucose) that are critical for normal neuronal differentiation during these developmental periods. Early-life iron deficiency anemia causes a 14 % reduction in hippocampal size (Rao, Tkac, Schmidt, & Georgieff, 2011) and abnormal CA1 dendritic structure accompanied by impaired synaptic transmission (Brunette, Tran, Wobken, Carlson, & Georgieff, 2010; Jorgenson, Sun, O'Connor, & Georgieff, 2005; Jorgenson, Wobken, & Georgieff, 2003). The observed abnormal hippocampal electrophysiology is consistent with the role of iron in NMDA-dependent hippocampal plasticity (Muñoz et al., 2011). Additionally, the ID hippocampus shows evidence of delayed maturation of pyramidal neurons as exemplified by reduced levels of doublecortin (Dcx), a microtubule-associated protein expressed in differentiating neurons (Francis et al., 1999), as well as delayed nuclear localization of nuclear neuronal marker NeuN (Tran et al., 2008). Moreover, Callahan, Thibert, Wobken, and Georgieff (2013) used biomarkers that define the opening and closing of developmental sensitive periods during hippocampal development and showed that fetal–neonatal iron deficiency delays the opening of the period, a response that may be considered adaptive. Nevertheless, iron deficiency also resulted in earlier closure of this critical developmental window, a finding consistent with loss of neural plasticity potential. Collectively, these studies show that early-life iron deficiency anemia impairs hippocampal differentiation and function. These alterations likely form the molecular basis underlying the abnormal hippocampal-dependent learning and memory behaviors seen while individuals are ID.

Chronic effects beyond the period of iron deficiency anemia

The long-term learning disability in FID humans and animal models suggests a persistently abnormal neural circuitry. This persistence can be due to two possibilities that are not mutually exclusive. One possibility is that having failed to construct the hippocampus and establish its connections normally during the acute phase of iron deficiency anemia (see above) the structural deficits in these neural circuits remain into adulthood and contribute to abnormal function. This hypothesis is supported by the findings of abnormal microstructure (Brunette et al., 2010) and loss of hippocampal volume in FID rats (Rao, Tkac, Schmidt, & Georgieff, 2011). A second possibility is that genes that mediate synaptic plasticity have been dysregulated through epigenetic modification of chromatin (see below). Although having a similar number of parvalbumin-antigen (PV+) cells to IS control, the FID hippocampus shows less PV mRNA level expression (Callahan et al., 2013). Lower parvalbumin expression may contribute to impaired plasticity in FID rat by reducing synaptic efficacy (Jiang et al., 2004) and dampening electrophysiologic long-term potentiation (LTP) (Jorgenson et al., 2005; Pisansky et al., 2013). These observations are consistent with reduced brain-derived neurotrophic factor (BDNF) signaling (see below) in the FID hippocampus (Grosse

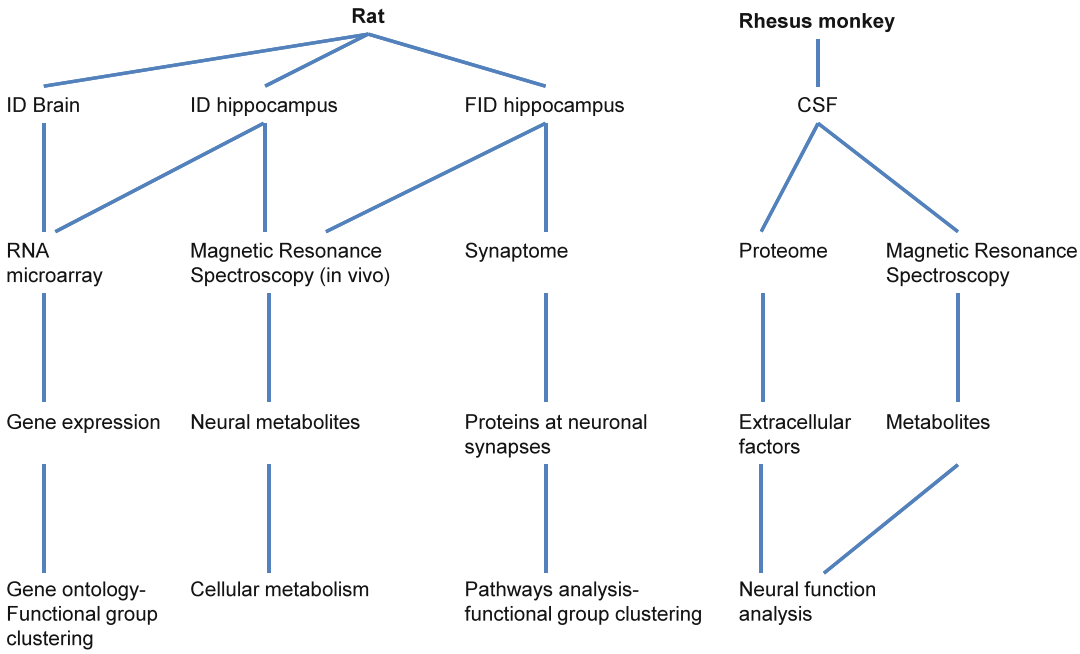

Fig. 15.2 Systems analyses of early-life iron deficiency models. Early-life iron deficiency can be induced by dietary manipulation (i.e., low-iron diet) in rats and by maternal stress during pregnancy in rhesus monkey. Whole brain or hippocampus from rats and cerebrospinal fluid from monkeys were analyzed by high-throughput technologies to identify system changes resulting from early-life iron deficiency

et al., 2005; Marty et al., 1996; Tran et al., 2009) and may well explain the underlying mechanisms behind long-term learning and memory impairments in FID rats (Schmidt et al., 2007) and mice (Fretham et al., 2012).

Early-life iron deficiency anemia alters gene expression in brain

The impact to society with respect to early-life iron deficiency anemia is the residual effect that appears to last well beyond the period of ID in spite of treatment. Although well documented in both humans and preclinical models, the mechanisms underlying the behavioral effects are only now being understood. To gain insight into the molecular underpinnings, systems level analyses (e.g., RNA microarray, proteome, metabolome, Fig. 15.2) were performed in ID and FID rodents, which identified changes throughout the brain (Clardy et al., 2006), particularly in salient pathways/networks involved in hippocampal energy metabolism, neuronal differentiation and synaptic plasticity (Carlson et al., 2007; Rao et al., 2003, 2007; Tran, Dakoji, Reise, Storey, & Georgieff, 2013).

Acute effects during iron deficiency anemia

Clardy and colleagues (2006) performed gene expression microarray analysis and found that early iron deficiency anemia alters gene expression in the whole brain of the ID rodent. Likewise, with a focus on the developing hippocampus, Carlson and colleagues (Carlson, Magid, Petryk, & Georgieff, 2008; Carlson et al., 2007) used a similar approach to identify iron deficiency-altered gene expression in non-anemic ID hippocampus and found alterations to notable networks centered on amyloid precursor protein and the mammalian target of rapamycin (mTOR). The amyloid precursor protein gene network has been implicated in cytoskeletal remodeling, cell motility and growth cone formation during hippocampal development (Guénette et al., 2006; Ikin, Sabo, Lanier, & Buxbaum, 2007). The mTOR

pathway integrates external metabolic conditions such as nutrient availability and growth factors necessary for the synaptic maturation and plasticity in the hippocampus (Schratt, Nigh, Chen, Hu, & Greenberg, 2004; Tang et al., 2002). Indeed, subsequent studies of hippocampal gene expression during and after iron deficiency corroborate these system level findings and more importantly demonstrate that these effects are specifically due to the loss of neuronal iron (Carlson et al., 2009; Fretham et al., 2012). Additionally, using the novel technology of high field magnetic resonance spectroscopy, Rao and colleagues (2003) found that iron deficiency profoundly alters neural metabolism in the developing rat hippocampus. Coe and colleagues used a proteomic approach to identify potential biomarkers altered by iron deficiency in the cerebrospinal fluid of ID baby rhesus monkey (Patton et al., 2012). They found changes in expression of factors critical for neuronal migration and synaptogenesis. Collectively, a systems analysis approach linking iron deficiency to gene expression, neuronal metabolites, and extracellular factors may ultimately establish a reliable and readily assessable biomarker or set of markers for clinical identification of early brain iron deficiency prior to the onset of anemia. Prognostic biomarkers of brain iron status could be important to prevent the long-term cost of early-life iron deficiency.

Targeted gene expression analysis also identified diminished hippocampal function of BDNF in the rodent models of fetal–neonatal iron deficiency (Blegen et al., 2013; Fretham et al., 2012; Tran et al., 2008, 2009). BDNF is a complex gene with multiple mRNA variants containing a common protein-encoding region (Aid, Kazantseva, Piirsoo, Palm, & Timmusk, 2007; Timmusk et al., 1993). BDNF regulates multiple aspects of hippocampal differentiation and function, particularly learning and memory (Heldt, Stanek, Chhatwal, & Ressler, 2007; Hennigan, O'Callaghan, & Kelly, 2007; Korte et al., 1995). Fetal and neonatal environments affect BDNF expression. For instance, an enriched environment has beneficial effects on hippocampal function and increases BDNF expression, whereas an adverse environment impairs memory function and lowers BDNF expression (Branchi, Francia, & Alleva, 2004; Pham, Winblad, Granholm, & Mohammed, 2002). Neural activity such as induction of LTP, a cellular phenomenon associated with memory formation, in the rodent hippocampus rapidly increases *Bdnf* mRNA levels (McAllister, Katz, & Lo, 1999; Patterson, Grover, Schwartzkroin, & Bothwell, 1992). In addition, early iron deficiency anemia also causes dysregulation of the insulin-like growth factor (IGF) signaling (Tran, Fretham, Wobken, Miller, & Georgieff, 2012). Given that IGF signaling is critical for neuronal growth, survival and differentiation, altered IGF function during a period of iron deficiency may underlie the smaller hippocampal volume observed in ID rats (Rao et al., 2011).

Chronic effects beyond the period of iron deficiency anemia

The long-term learning disabilities in FID humans and animal models suggest persistent dysregulation of genes critical for synaptic function. Coe and colleagues identified persistent changes in cerebrospinal fluid in a monkey model of early iron deficiency that are critical for neuronal migration, synaptogenesis, and myelination (Geguchadze et al., 2008; Patton et al., 2012). These findings suggest sustained effects of early ID on neuronal connectivity and function. Likewise, Georgieff and colleagues investigated these long-term effects and found evidence for alterations in gene expression as well as protein levels at the synapses in FID adult rat hippocampus (Tran et al., 2009, 2013). A notable long-term change in gene expression is the suppression of hippocampal *Bdnf* and its high affinity receptor TrkB in the FID adult rat (Tran et al., 2009). The reduction of BDNF expression may stem from a less neural activity-driven transcriptional response or a more permanent change in its regulation such as epigenetic modification of chromatin (e.g., DNA methylation, histone methylation) at the BDNF locus (Blegen et al., 2013; Roth, Lubin, Funk, & Sweatt, 2009; Tran, Kennedy, Lien, Simmons, & Georgieff, 2014). With regard to epigenetic modification, further investigation focused on the effects of iron deficiency on

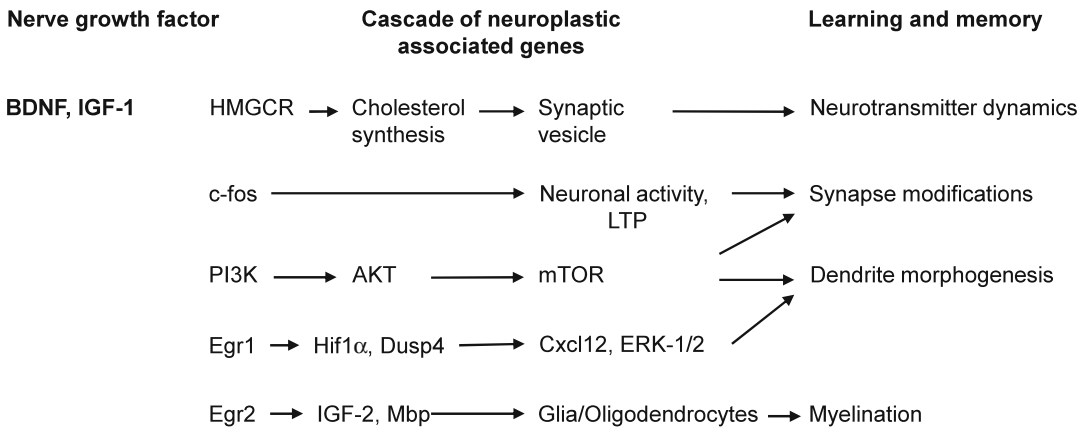

Fig. 15.3 Early-life iron deficiency alters gene signaling cascades that regulate learning and memory in the adult rat hippocampus

expression and function of a family of JmjC-domain containing proteins (JARIDs), which require iron as a cofactor for their catalytic activity to remove methyl groups from histones, which may provide a direct link between iron deficiency and epigenetic modification of genes. Early iron deficiency-induced long-term BDNF suppression leads to lower expression of BDNF-activity dependent gene cascades (Fig. 15.3). For instance, in the postnatal brain BDNF regulates neuronal HMGCR, a rate-limiting enzyme in cholesterol synthesis that facilitates synaptic vesicle formation (Suzuki et al., 2007). Consistent with lower neuronal BDNF activity, the level of HMGCR was decreased in P65 FID hippocampus (Tran et al., 2009). Combined with lower expression of synaptic proteins involved in synaptic vesicle dynamics (Carlson et al., 2007; Tran et al., 2013), this finding may explain the reduced synaptic efficacy seen in adult hippocampal slice preparations following recovery from fetal–neonatal iron deficiency (Jorgenson et al., 2005). Likewise, expression of activity-dependent immediate early transcription factors (e.g., c-fos, Egr-1, and Egr-2) that facilitate LTP in the hippocampus (Alder et al., 2003; Rössler & Thiel, 2004) are reduced in FID rat hippocampus (Tran et al., 2009). Given that LTP is widely accepted as a cellular substrate for learning and memory (Malenka, 2003), lower expression of these genes in FID rats may in turn reduce formation of LTP (Miyamoto, 2006), expression of BDNF (Dong, Wu, Fan, Xu, & Zhang, 2006), Hif1α and Dusp4 (Berasi et al., 2006; Sperandio et al., 2009), thereby leading to a less plastic and lower capacity hippocampus in FID rats.

Potential Adjunctive Therapies to Iron

The persistent neurobehavioral abnormalities of early-life iron deficiency anemia, in spite of prompt iron treatment in humans and in preclinical models, reflect a fundamental problem of iron treatment alone; it is not fully effective. Thus, there is a real need to identify evidence-based adjunctive therapy to complement iron treatment. The adjunctive treatments listed below have been shown in either preclinical or clinical trials to result in long-term improvements specific to cognitive function or socio-emotional behaviors in typically and atypically developing states. Furthermore, many of these treatments are hypothesized to improve brain function through altered expression of the same neurotrophic factors that are dysregulated by fetal–neonatal iron deficiency. It is worth noting that the preclinical studies that have demonstrated behavioral improvements and/or changes in expression patterns of neurotrophic factors following application of these treatments are often performed

using rodent models of developmental insults that share many of the same characteristics of human fetal–neonatal iron deficiency.

Given the long-term dysregulation of genes critical for neuroplasticity in early-life ID animals, particularly BDNF, interventional strategies that improve cognitive function accompanied by enhanced BDNF activity such as enriched environment, exercise, or selective serotonin reuptake inhibitors (Russo-Neustadt, Beard, Huang, & Cotman, 2000) may be useful as therapeutic approaches to treat long-term cognitive dysfunction of fetal–neonatal iron deficiency.

Methyl diets/Choline

Studies in animal models of early-life insults identify choline, a readily available and essential nutrient involved in early brain development, as a potential supplementary nutrient to reverse the cognitive dysfunctions associated with developmental disorders such as fetal alcohol syndrome and Down's syndrome (Moon et al., 2010; Ryan, Williams, & Thomas, 2008) as well as adulthood disorders including age-related dementia, brain injury, and seizures (Guseva, Hopkins, Scheff, & Pauly, 2008; Holmes et al., 2002; Meck, Williams, Cermak, & Blusztajn, 2007). The enhanced cognitive function resulting from choline supplementation is accompanied by increased hippocampal neurogenesis (Glenn et al., 2007), reduced thresholds for LTP (Pyapali, Turner, Williams, Meck, & Swartzwelder, 1998), increased dendritic arborization (Li, 2004) and increased expression of neurotrophic factors such as BDNF (Glenn et al., 2007). Recently, Kennedy and colleagues (2014) demonstrated that choline supplementation during mid- to late gestation can rescue the long-term cognitive impairment in FID adult rats following fetal–neonatal iron deficiency. Thus, choline supplementation is an attractive therapeutic strategy to complement iron treatment for early-life iron deficiency.

Enriched environment

An enriched environment can also dramatically alter brain development and improve function in preclinical models. Environmental enrichment in rodent experiments often refers to a larger living space, larger social groups, introduction of novel or interactive objects, and exercise (Van Praag, Kempermann, & Gage, 2000). However, because exercise can have beneficial effects that are dissociable from environmental enrichment (Olson, Eadie, Ernst, & Christie, 2006), exercise is considered as a separate, but potentially additive (Fabel et al., 2009), adjunctive treatment and discussed in the next section. Extended periods of environmental enrichment (6–8 weeks after weaning) improve performance on hippocampal-dependent memory tasks (Duffy, Craddock, Abel, & Nguyen, 2001; Wainwright, Lévesque, Krempulec, Bulman-Fleming, & McCutcheon, 1993), reduce anxiety (Schrijver, Bahr, Weiss, & Würbel, 2002), and increase sociability (Morley-Fletcher, Rea, Maccari, & Laviola, 2003) in rats. Furthermore, environmental enrichment can reverse some of the socio-emotional and cognitive deficits observed in rodent models of developmental insults including autism spectrum disorders (Schneider, Turczak, & Przewłocki, 2006), fragile X syndrome (Restivo et al., 2005), prenatal stress (Morley-Fletcher et al., 2003), and fetal alcohol syndrome (Hannigan, O'Leary-Moore, & Berman, 2007).

Exercise

Regular exercise has long been known to boost cognitive abilities and studies have demonstrated that exercise can improve cognitive performance in rodent models of neurological damage including traumatic brain injury (Griesbach, Hovda, & Gomez-Pinilla, 2009) and stroke (Luo et al., 2007) as well as following prenatal insults such as fetal alcohol syndrome (Boehme et al., 2011). In these studies, voluntary exercise has been shown to improve both spatial (Aguiar et al., 2011; Griesbach et al., 2009; Luo et al., 2007; Vaynman, Ying, & Gómez-Pinilla, 2004) and recognition (Hopkins & Bucci, 2010) forms of memory in rats. There is additional evidence that exercise can decrease anxiety-like behavior (Hopkins & Bucci, 2010). The beneficial effects of exercise have been attributed to changes in hippocampal BDNF based on evidence that voluntary exercise increases expression of BDNF in the hippocampus and this increase is either asso-

ciated with (Aguiar et al., 2011; Hopkins & Bucci, 2010) or required for (Griesbach et al., 2009; Vaynman et al., 2004) improvements in memory. Consistent with these findings, exercise increases peripheral levels of BDNF in humans which is associated with improved learning (Winter et al., 2007).

Mother–infant centered therapies

One adjunct treatment that has already been demonstrated to improve outcomes of ID human infants is behavioral home intervention (Lozoff et al., 2010). In this study, 6- or 12-month-old ID infants were enrolled in a program that involved weekly visits from trained monitors who would work with the ID infants and their caregivers to improve infant–caregiver interactions and provide support for any infant development-related concerns. Compared to a control group which only received weekly surveillance visits, the home intervention normalized raw mental development scores to the level of IS individuals at follow-up evaluations 6 and 12 months later. Measures of positive socio-emotional behavior were similarly improved in ID infants receiving intervention. Whereas behavioral scores in the ID surveillance-only group decreased at follow-up evaluations, home intervention reversed this effect, resulting in a more developmentally appropriate increase in positive socio-emotional scores over time. However, unlike raw mental scores, behavioral scores were lower in ID infants at all timepoints regardless of treatment, suggesting a need for earlier intervention or additional adjunct treatments.

Summary

Early-life iron deficiency is highly prevalent around the world and confers a major risk factor to the developing brain. The literature on outcomes of ID neonates and children strongly supports the hypothesis that neurobehavioral outcomes are a function of the timing, degree and duration of iron deficiency. These neurobehavioral outcomes occur acutely during the period of iron deficiency, but also result in long-term residual deficits in spite of the (rapid) correction of the anemia. It is the latter that is the greatest cost to society in terms of the extra support, loss of job and educational potential, and medical care that FID individuals may need. An extensive literature of preclinical models of early-life iron deficiency has identified multiple iron-containing and iron-dependent systems of the young brain that are particularly at risk: monoamine neurotransmission, myelin, neuronal migration, and neuronal differentiation, particularly in the hippocampus. Each remains abnormal after correction of the experimentally induced iron deficiency. Recent studies have confirmed that the lack of iron in the brain, as opposed to the anemia, is responsible for the neurocognitive deficits and that the long-term changes are explained in part by alterations to brain structure as well as gene regulation. Given the failure of iron repletion to restore complete brain health, two agendas going forward will be identification of measurable biomarkers of brain iron deficiency prior to the onset of anemia and testing of adjunct therapies that focus on redressing the basic biology altered by early-life iron deficiency.

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Observing the Fetus' Behavior to Assess Health: The Behavior of the Human Fetus in Response to Maternal Alcohol Consumption

16

Peter G. Hepper

Abstract

This chapter reviews studies that have examined the behavior of the human fetus in response to maternal consumption of alcohol. While focussing on how alcohol influences the behavior of the fetus this is used to illustrate how studies of prenatal behavior can contribute to the assessment of the health and well-being of the fetus. I first review the link between the behavior of the fetus and the functioning of its brain and briefly consider the effect of environmental agents upon the behavior of the fetus. The effects of alcohol on the individual after birth are considered and then studies which have assessed the behavior of the fetus in response to acute and chronic alcohol consumption are reviewed. The insights these observations provide into the mechanism of alcohol induced effects are discussed and how these can be used to inform pregnant women considered.

Keywords

Alcohol • Fetus • Behavior • Brain • Startle • Habituation • Breathing • Behavioral states

The aim of this chapter is to review studies that have examined the behavior of the human fetus in response to maternal consumption of alcohol. This review is set in the more general context of illustrating how studies of behavior during the prenatal period can contribute to the assessment of the health and well-being of the fetus.

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Human Fetal Behavior

Scientific interest in the behavior of the fetus can be traced back to the beginning of the twentieth century (see review, Hepper, 1992). However, it was only after the introduction of ultrasound technology to monitor the fetus during pregnancy in the late 1970s/early 1980s, which enabled the fetus to be viewed in great detail, that studies began to explore the behavior of the fetus in depth (see reviews Nijhuis, 1992). These studies

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continue today and, as a global conclusion, have revealed just how significant the prenatal period is for the development of the fetus (see this volume).

Explorations of the behavior of the fetus have been conducted with three main, non-mutually exclusive, aims. Initial studies (e.g., de Vries, Visser, & Prechtl, 1982, 1985), and indeed the majority since, have focussed on describing the behavior of the fetus. A second, and related goal, has been to investigate the functions that fetal behavior may serve to promote both development and survival. However, less attention has been paid to this aim (Hepper, 2015). Finally, studies have been undertaken to use fetal behavior as an indicator of well-being and health (Hepper, 1995a): it is this aim that is the focus of this chapter.

If the behavior (throughout this chapter I use the term "behavior" to cover both the fetus' spontaneous and elicited movements) of the fetus is to be used as an indicator of well-being or distress then a comprehensive description of normal behavior is required (Visser, Mulder, & Ververs, 2010). This can then be used as a "gold-standard" against which the behavior of individual fetuses is compared. Deviations in the fetus' behavior from the "norm" may be used as an indication that something is amiss (Hepper, 1995a). Of particular interest has been the potential use of the fetus' behavior to provide information on the functioning of the brain (Hepper, 1995a).

Fetal Behavior and Brain Function

It has been argued, and is now generally accepted, that as the behavior of the fetus is a reflection of the functioning and integrity of the central nervous system, the behavior of the fetus can be used to provide information on the health of the brain (e.g., Hepper, 1995a; Visser et al., 2010). The well-being of the brain remains difficult to evaluate, even in adults, and behavioral measures provide some of the most comprehensive and useful tools to evaluate brain functioning. Assessing the health of the fetus' brain is made even more problematic due to its inaccessibility (i.e., its location within the mother's womb precluding direct access). However, the behavior of the fetus may be observed and this presents an opportunity to evaluate brain functioning. Although there are other techniques that may be used to assess the fetus' brain, these have significant disadvantages compared to the observation of fetal behavior.

Techniques that evaluate either chromosomal or genetic composition, while accurately determining the presence of a genetic or chromosomal anomaly, may not directly assess the influence of this upon brain function and/or behavior. Take, for example, Trisomy 21, Down syndrome. Cellular analysis very accurately detects the presence of an extra chromosome at locus 21. However, this does not provide an indication of how the individual's brain will perform other than in the most general terms. Individuals with Down syndrome may present within a range of cognitive abilities, and the specific individual outcome is not able to be determined from the detection of an extra chromosome alone. Similar problems exist with the detection of structural anomalies, for example, through their visualization with ultrasound or MRI. While gross structural brain abnormalities may be detected, smaller anomalies, which may be meaningful in terms of functional outcome, may not be detected. Some easily detectable gross anomalies may have limited meaning for outcome (e.g., choroid plexus cysts). For those structural abnormalities detected, their effect on the functioning of the brain, other than at the gross general level, will be difficult to predict. In recent years, techniques that assess the functioning of the brain, (e.g., fMRI) have begun to be used (e.g., Messing-Junger et al., 2009; see also Chaps. 20–23). While these may elucidate differences in the functioning of areas of the brain between fetuses, how any detected differences relate to behavioral performance requires further exploration. In contrast to these techniques, the behavior of the fetus more directly reflects the current functional capability of the brain. With a "direct" link between the functioning of the fetus' brain and the production of behavior (Hepper, 1995a), the behavior of the fetus provides an indication of how the brain is actually operating at the time of observation, and hence provides a valuable opportunity to assess

brain function (Hepper, 1995a; Visser et al., 2010).

Problems Evaluating the Effects of Prenatal Exposure to Adverse Environmental Agents

The ability to observe, via the behavior of the fetus, the functioning of the brain is highly advantageous for assessing the potential impact of adverse environmental agents, ranging from teratogens (e.g., cigarette smoke) through maternal disease (e.g., diabetes) to maternal psychological state (e.g., depression/anxiety). The evaluation of the impact of exposure to such agents in pregnancy is problematic as individuals may not know whether they have been exposed nor the extent, amount and duration of exposure to which they have been subjected. Moreover, individuals may be reluctant to admit their exposure to substances that are known to be harmful (e.g., cigarettes, drugs) but are consumed as a matter of personal choice. The problems for evaluating the effects of environmental agents arising from this lack of information surrounding exposure levels is further complicated by the fact that the effect of many environmental agents is influenced by individual factors within the mother and fetus. The same exposure to a particular agent may have different outcomes in different individuals as a result of unique maternal and/or fetal characteristics. Thus, predicting or evaluating the outcome arising from exposure to potentially harmful environmental agents based on reported exposure levels is extremely problematic. Observation of the behavior of the fetus on the other hand avoids these difficulties. As one observes behavior, using this as the outcome measure for the effect(s) of any particular agent on the brain, information on exposure is not relevant. The greater the deviation in behavior observed, the greater the impact of the agent independent of information on exposure levels. One environmental agent, which presents all these problems in evaluating exposure, and may cause significant damage to the fetus' brain, is alcohol.

Prenatal Exposure to Alcohol

In 1968, Lemoine, Harrousseau, Borteyru, and Menuet reported that children of mothers who had consumed large amounts of alcohol during pregnancy exhibited unusual physical and neurobehavioral abnormalities. They noted that these children displayed a particular constellation of features comprising central nervous system dysfunction, growth retardation both prenatally and postnatally, and a characteristic facial appearance. The term, Fetal Alcohol Syndrome (FAS), was subsequently proposed to describe this set of abnormalities (Jones & Smith, 1973). As research investigated further the effects of maternal alcohol consumption during pregnancy on the fetus it was realized that FAS was at the most severe end of a range of effects that may arise (Riley & McGee, 2005). A variety of anomalies affecting physical, organ, growth, behavioral or neuropsychological parameters, occurring either uniquely or in combination have been reported, often arising from lower levels of alcohol consumption. The term applied to capture this range of deficits is Fetal Alcohol Spectrum Disorders (FASD) (Riley, Infante, & Warren, 2011). The actual symptoms exhibited by the child following exposure to prenatal alcohol vary between individuals and are influenced by the individual characteristics of the mother and the fetus and also when during gestation exposure occurred, for how long, and at what level of exposure (Goodlett, Horn, & Zhou, 2005). One of the most common effects of prenatal alcohol exposure is brain (central nervous system) dysfunction (Riley et al., 2011). This may result in problems in the individual's learning, cognitive, emotional, perceptual, and motor performance. As a result of these deficits, individuals may suffer social and behavioral problems and the child may exhibit difficulties at school. Social problems, including alcohol and drug problems, inappropriate sexual behavior may all occur and may lead to interactions with the criminal justice system (Streissguth, 1997). In summary, fetal exposure to alcohol as a result of maternal consumption may lead to a variety of neurobehavioral problems which will exert a significant adverse effect on the individual across his or her lifespan.

It is widely accepted that alcohol is a leading nongenetic cause of intellectual disability and behavioral problems in children (BMA, 2007). The actual incidence of FAS and FASD is difficult to determine due to a lack of diagnostic criteria and services to assess individuals (BMA, 2007). In the UK there are no reliable figures for the incidence of FAS and FASD. Moreover, the incidence of these disorders is significantly influenced by the population under study (BMA, 2007). For example, estimates of the incidence of FASD range from 10/1000 live births in Canada to as high as 89/1000 live births in Cape Province of South Africa. In the absence of more accurate data conservative worldwide estimates of FAS suggest an incidence of about 1/1000 live births and for FASD 1/100 live births (BMA, 2007).

As well as exposure to alcohol causing considerable and significant problems for the developing child and their caretakers, the economic cost to Society as a whole is also substantial. Estimates based on 2002 figures placed the lifetime medical cost of a child with FAS in the USA as 2.9 million USD (Lupton, Burd, & Harwood, 2004). In England recently a 16-year-old, who suffered severe brain damage as a consequence of the mother consuming alcohol while she was pregnant with the child, was awarded £500,000 compensation (Templeton, 2014). Although caution must be taken in extrapolating from single cases, if this were to be applied across all affected live births in the UK (1 % [affected by FASD] of live births [812,970 in 2012]) this could result in an additional cost of over £4 billion/year.

Thus, prenatal exposure to alcohol may result in significant lifelong problems for those affected, with the most prevalent aspect being cognitive and behavioral problems. It also has an impact on the carers of those affected and the wider society. Most alarming is perhaps the fact that these effects are entirely and completely preventable.

Fetal Behavior Following Alcohol Exposure

There is an extensive literature documenting the effects of maternal alcohol consumption during pregnancy on the child after birth (BMA, 2007; Riley et al., 2011). There are far fewer studies that have examined the effects of alcohol before birth. Thus, while the postnatal outcome of prenatal exposure to alcohol on the individual has been well-researched, very little insight has been gained into how alcohol affects the individual at the time of exposure. Such information is crucial if a complete understanding of how alcohol affects the brain and subsequent behavior is to be achieved, and advances are to be made in the diagnosis and treatment of the effect(s) of prenatal alcohol exposure.

Those studies that have examined how maternal alcohol consumption influences the behavior of the human fetus are now appraised. The review is divided into three sections based upon the type of study: case studies; studies observing behavior where alcohol is present in the mother's system ["acute" effects of alcohol]; and studies observing behavior in fetuses of mothers who drink but do not have alcohol in their system at the time of study ["chronic" effects of alcohol]. The review of these studies will demonstrate how the analysis of the behavior of the fetus can be used to overcome some of the problems of evaluating the effects of environmental agents on the fetus and advance our understanding of how these agents exert their effects.

Case Studies

Case studies provide a valuable source of information as they frequently involve instances of rare occurrences or extreme exposures, providing a unique insight into the subject under study. For cases involving prenatal alcohol consumption they involve high levels of consumption which would not be feasible to administer in a research study, but which are found amongst the population of pregnant women. Of course, as studies of single individuals, caution must be exercised in interpreting these studies but they do provide significant information on the effects of high levels of alcohol on the fetus and her brain and behavior.

Castillo, Devoe, Ruedrich, and Gardner (1989) report an observation of a fetus used as his own control. The fetus was first observed when the

mother had a large amount of alcohol in her system (a blood alcohol level of 322 mg/dL, the legal limit for driving in the UK is 80 mg/dL) and then observed for a second time, 24 h later when the mother's blood alcohol level was less than 10 mg/dL. The observations were conducted at 37 weeks of gestation. The mother was reported as having a history of heavy drinking throughout her pregnancy. The fetus was observed for 60 min on each occasion and both breathing and body movements were observed. When the mother was intoxicated the fetus exhibited significantly fewer body and breathing movements than when observed 24 h later when the mother was not intoxicated.

A second case study recorded the behavioral state(s) of a single fetus that had been exposed to heavy maternal alcohol consumption (Mulder, Kamstra, O'Brien, Visser, & Prechtl, 1986). Behavioral states are first observed in the fetus at 36 weeks of gestation (Nijhuis, Prechtl, Martin, & Bots, 1982). They are defined through the use of three behavioral parameters: fetal heart rate (Pattern A, B, C, D); fetal body movement (present, absent); and fetal eye movements (present, absent). Four states are identified: 1F (quiescence), 2F, 3F, 4F (active). The emergence of behavioral states is thought to be important as it marks a significant development in the maturation of the fetus' brain, reflecting greater integration and functioning between different brain regions (Nijhuis et al., 1982). Mulder et al. (1986) undertook observations of a fetus whose mother had consumed a minimum of ten glasses of beer per day during the first trimester and between two and ten glasses per day thereafter. The fetus was observed for approximately 2 h at 38 weeks of gestation and again at 40 weeks of gestation, following the mother's earlier admission to hospital at 36 weeks of gestation. The usual quick transitions observed as the fetus moves from one state to another (Nijhuis et al., 1982), with very little time spent in no state, were not observed. In this case the fetus spent a large amount of time in no particular state. The fetus also jumped directly from state 1F to state 4F. This is not the normal pattern of orderly transition between states that is usually seen where the fetus moves from state 1F to 2F and then to 4F. The authors suggest this unusual pattern of responding may be a result of the heavy exposure to alcohol during pregnancy and/or a withdrawal effect as a result of the mother being unable to access alcohol during her stay in hospital.

These case studies clearly indicate that alcohol disrupts the normal pattern of behavior exhibited by the fetus. In one instance movement was "suppressed" and in a second the normal neurobehavioral coordination seen in behavioral states was disturbed.

The Acute Effects of Maternal Alcohol Consumption on the Behavior of the Fetus

One approach to the study of how alcohol affects the fetus has been to ask the mother to consume some alcohol and then observe the behavior of the fetus following consumption. These studies have been undertaken in late gestation, usually the last month of the pregnancy, and have focussed their attention around the recording of fetal behavioral states and/or fetal breathing movements.

Fetal breathing movements may be observed from 10 to 12 weeks of gestation. Initially, breathing movements are infrequent but display an increase in incidence from 26 weeks of gestation to 36 weeks of gestation. The most rapid increase in their occurrence occurs between 26 and 30 weeks of gestation (James, Pillai, & Smoleniec, 1995). Initially, breathing movements occur regularly, if infrequently, but become organized into bouts of activity and inactivity after 28 weeks of gestation (Cosmi, Anceschi, Cosmi, Piazze, & La Torre, 2003).

Studies that have examined fetal breathing movements following the administration of alcohol to the fetus via maternal consumption have all reported the same effect. As soon as the mother drank the alcohol there was an immediate decrease in the incidence of fetal breathing movements and these movements were completely abolished 30–40 min after initial consumption. These results were identical despite some slight differences between the studies. McLeod et al. (1983) observed fetuses, aged between 37 and 40 weeks of gestation, over a 3.5-h period following maternal consumption of 1.9 oz ethanol (40 %

alcohol, 80 proof) diluted in soda water. Fox et al. (1978) observed fetuses, aged between 37 and 39 weeks of gestation, for 60 min following maternal consumption of 1 oz of 80 proof vodka (40 % alcohol) in 90 ml of ginger ale. Akay and Mulder (1996) observed fetuses for about 2 h, between 37 and 40 weeks of gestation, following maternal consumption of two glasses of white wine. McLeod et al. (1983) continued their observations for 3 h after the mother consumed the alcohol and reported that the fetus' breathing movements were still suppressed when their observation period ended. At the conclusion of their observation period only 2 of 6 mothers had ethanol in their blood, and this was at a very low level (0.01–0.02 mg/ml). Comparing the results between studies, there is some suggestion of a dose dependent effect. McLeod et al. (1983) who asked their mothers to consume 1.9 oz of alcohol reported a longer period when movements were suppressed than Fox et al. (1978) who asked mothers to consume 1 oz of alcohol.

The effect that alcohol has in suppressing behavior may account for the disruption of behavioral states observed in fetuses aged 37–40 weeks of gestation after their mothers had been asked to consume two glasses of white wine (0.25 g ethanol/kg of maternal body weight; Mulder, Morssink, van der Schee, & Visser, 1998). Behavioral states became disrupted immediately upon consumption of the alcohol which may be largely due to the reduction in both breathing and eye movements, key parameters in the determination of behavioral states. Of particular note is the observation regarding the disruption of the fetus' eye movements (see below).

Studies examining the acute effects of alcohol consumption on the fetus reveal consistent results. There is an immediate effect of alcohol on the fetus which reduces its movements and these movements are completely abolished 30–40 min after consumption. The duration of the suppression of behavior appears to be dose-related with larger amounts of alcohol suppressing behavior for longer. Further, suppression continues even when there appears to be little alcohol remaining within the mother's body, evaluated from ethanol levels in maternal blood (McLeod et al., 1983).

The results from these studies highlight two important factors which emerge through the study of the fetus' behavior. First, alcohol induces effects in the fetus at low levels of exposure, as little as one glass of alcohol. Behavioral observations allow the effects of different amounts of alcohol exposure to be documented as they occur. Observations of behavior over time, following maternal consumption of alcohol, allow the time-course of the effects of alcohol to be elucidated. In doing so, a second key point emerges, namely that alcohol continues to exert an effect on the fetus even when it has been cleared from the mother's body. This is important as it indicates that the duration of exposure to alcohol experienced by the fetus is much longer than that experienced by the mother.

Observation of how the behavior of the fetus is affected by acute exposure to alcohol can be used to confirm the proposed physiology and pharmacology of the mechanisms of elimination of alcohol from the fetus' body.

Alcohol readily crosses the placenta and enters the fetal blood stream. Alcohol is not cleared from the fetal system (Brien, Loomis, Tranmer, & McGrath, 1983) but rather has to pass back into the maternal system for breakdown and elimination (Heller & Burd, 2014). Alcohol may act as a vasoconstrictor and slow the flow of alcohol passing from the fetus to the mother, especially in later pregnancy; this would prolong the exposure of the fetus to alcohol. The fetus is unable to break down alcohol. As such, alcohol may enter the amniotic fluid via urination or pulmonary activity providing a means of continued experience of alcohol by the fetus. Prior to 20 weeks the fetus can absorb alcohol through the skin but as the skin becomes keratinized around this age this route is unavailable after 24 weeks. The fetus swallows amniotic fluid from 15 weeks of gestation and will thus reexperience alcohol in the amniotic fluid through this route (Hepper, 1992). All of these mechanisms act to prolong the duration of exposure to alcohol by the fetus compared to that experienced by the mother. Following the time course of the behavior of the fetus after acute exposure to alcohol enables this to be observed.

The Chronic Effects of Maternal Alcohol Consumption on the Behavior of the Fetus

Studies reviewed in this section have identified women who drink, or have drunk, during their pregnancy and have examined the behavior of their fetuses comparing this to the behavior of fetuses whose mothers have not consumed alcohol. The studies described below were performed when there was no alcohol detectable in the mother's system as assessed by measurement of maternal breath alcohol levels at the time of the study. Hence, it is argued these studies examine a more "permanent" effect of alcohol on the fetus and its behavior. Studies control for other factors that might influence the behavior of the fetus such as maternal anxiety and stress (Van den Bergh, 1992) and smoking (Leader, 1987).

Startle Behavior

One aspect of the behavior of the fetus that has come under examination in these studies is the startle response of the fetus. The startle response may be defined as a rapid onset movement, which originates in the limbs and then involves the whole body. It is of short duration lasting about 1 s (de Vries et al., 1985). The spontaneous startle is one of the first movements exhibited by the fetus and is observed around 8 weeks of gestation (de Vries et al., 1982, 1985). The frequency of the startle rapidly increases in occurrence and peaks in frequency at 9 weeks of gestation, after which its incidence decreases and, after mid-gestation, it is rarely seen (de Vries et al., 1982, 1985). Due to the early appearance of the startle in the fetus' development, this response is thought to be one of the fetus' most basic movements and reflects the primitive organization of its nervous system. Its disappearance is linked to the advancing development and integration of the nervous system and brain which results in the fetus exhibiting more complex, organized, and sophisticated patterns of behavior. It is believed that the startle response is initially largely controlled by excitatory pathways and the development of inhibitory pathways contributes to its disappearance.

In later gestation, the startle response may be observed following fetal "stimulation" by an external stimulus. Identical in appearance to the spontaneous startle, the elicited startle occurs immediately after the presentation of stimulus (e.g., a loud noise). The onset and appearance of the elicited startle is largely determined by development of the sensory system involved in the detection of the eliciting stimulus. Thus, a startle response is first observed to a loud sound at around 24–26 weeks of gestation and as gestation progresses the response can be elicited by less intense stimuli, and more intense stimuli evoke a larger (greater movement amplitude) and faster (initiation of movement sooner after stimulus onset) response. This change in the response reflects the development of the fetus' auditory system which begins to function around 24 weeks of gestation and becomes more sensitive to sounds as gestation progresses (Hepper & Shahidullah, 1994). In contrast to the spontaneous startle which is endogenously generated, the elicited startle requires three components to operate: a functioning motor system to produce the response, a functioning sensory system to detect the stimulus, and a link between sensory and motor system. Alterations in the elicited startle response may be due to the lack of function in any one of these components.

Little, Hepper, and Dornan (2002) observed the incidence of spontaneous startles in fetuses aged 18–20 weeks of gestation over a 45-min period. They compared fetuses of mothers who drank during pregnancy (an average of 2.5 units of alcohol/week) with fetuses whose mothers did not drink. At the time of testing, none of the mothers had any alcohol in her system. It was found that fetuses who had been exposed to alcohol produced significantly more spontaneous startles than fetuses not exposed. In a follow-up study (Hepper, Dornan, & Little, 2005), the researchers documented the longitudinal development of spontaneous startle behavior from 20 to 35 weeks of gestation. Again the number of spontaneous startles exhibited by fetuses of mothers who drank alcohol (consuming an average of 4.2 units of alcohol/week), but had none in their system at the time of testing, was compared to the number exhibited by fetuses of

mothers who did not drink. The same fetuses were observed at 20, 25, 30, and 35 weeks of gestation. At all ages studied, more spontaneous startles were observed in fetuses who had been exposed to alcohol. However, as gestation advanced the number of startles exhibited by the fetuses exposed to alcohol decreased, and approached the level of startle behavior exhibited by fetuses not exposed to alcohol at 35 weeks of gestation. Although decreasing, the number of spontaneous startles was still higher than in fetuses not exposed to alcohol at 35 weeks of gestation, when the study stopped.

Little et al. (2002) studied elicited startles using a Corometrics vibro-acoustic stimulator to elicit the startle response in the fetus. This hand held device produces a "sound" of approximately 75 Hz (20–90 Hz) at an intensity of 82 dB measured at 1 cm. The head of the device, which vibrates as well as acting as a speaker, is placed on the mother's abdomen, usually over the head of the fetus, to provide the stimulation. The startle response of fetuses of mothers who drank (an average of 2.5 units of alcohol/week) was compared to that of fetuses whose mothers did not drink alcohol, at 25 weeks of gestation. When stimulated, fetuses of mothers who did not drink exhibited significantly more startles than fetuses of mothers who did drink.

Hepper, Dornan, Lynch, and Maguire (2012) examined the influence of maternal consumption of alcohol on the elicited startle in a longitudinal study. Fetuses were either exposed to alcohol via their mother's drinking (an average of 10 units of alcohol/week) or had no exposure to alcohol. Fetuses were observed at 29, 32, and 35 weeks of gestation and the startle elicited by the presentation of a 2-s duration 70–250 Hz pink noise (90 dB at 1 cm) presented via a loud speaker placed on the mother's abdomen over the fetus' head. The strength of the startle response was evaluated and ranged from 0, no response, to 4, a rapid large amplitude movement of all observed body parts (arms, head, body). At 29 weeks of gestation a weaker startle response was observed in those fetuses who had been exposed to alcohol compared to those fetuses who had not been exposed. There was no difference between the two groups of fetuses at either 32 or 35 weeks of gestation. One important aspect to this study was that the motor responsiveness of the fetus also was examined to determine whether the difference in the startle response was caused by a difference in motor abilities. This was particularly important given the suppression of fetal breathing movements observed in studies reported earlier. The study found no differences between the number of spontaneous movements exhibited by the fetuses in each group, suggesting that a difference in motor capability was not the reason for the difference in elicited startles observed.

Taken together the results from studies that have examined the effect of alcohol on the fetal startle response indicate that exposure to alcohol leads to a delay in the fetus' neurobehavioral development. Spontaneous startles, which as a primitive neural pattern should be rare in occurrence, are preserved in fetuses exposed to alcohol. The onset of elicited startles is delayed in fetuses exposed to alcohol. It is most likely that the effects are induced by the effect of alcohol delaying neural maturation. One effect of alcohol exposure on individual nerves is that it delays the growth of dendrites and myelination of the nerves (Goodlett et al., 2005). This may contribute to the delay in the fetus' startle responses observed here.

There was no alcohol in the mother's system at the time of testing and hence the effects are likely due to a "chronic" affect. Although as discussed earlier, the amount of alcohol in the fetal system is difficult to determine.

One notable observation from these studies was that there was no correlation between the number of startles the fetus exhibited and the amount of alcohol drunk by the mother. This supports previous observations that, because of individual differences in mothers and in fetuses, the amount of exposure is not that predictive of outcome (Mattson & Riley, 2011). This also highlights one of the benefits of observing behavior as a measure of the effects of alcohol consumption on the fetus. The behavior of the fetus directly reflects the effect of alcohol on the fetus' brain and thus provides a much more reliable assessment of the effects of alcohol than information on the amount of alcohol consumed.

Habituation

A further task that has been used to assess the effects of alcohol on fetal behavior and brain function is that of habituation. Habituation is considered to be a form of learning and can be defined as the decrement in response to repeated presentation of the same stimulus (Hepper & Leader, 1996). In the fetus, habituation is usually examined using the repeated presentation of a discrete auditory stimulus and the fetus' movement and/or heart rate observed in response to each stimulus presentation. The fetus exhibits a large movement or change in heart-rate upon the first presentation of the stimulus but as the stimulus is repeated at regular intervals its response wanes until it no longer responds. The number of stimulus presentations until no further responding is observed is recorded as a measure of habituation. Habituation is considered to provide a valuable tool to assess how the fetus' brain is functioning as it requires an undamaged and operational central nervous system, including the cortices, to perform normally (Hepper & Leader, 1996). Studies have demonstrated that fetal habituation performance is adversely affected by: environmental conditions such as maternal smoking (Leader, 1987), fetal conditions such as Trisomy 21 (Hepper & Shahidullah, 1992), and maternal conditions such as diabetes (Doherty & Hepper, 2000).

Hepper, Dornan, and Lynch (2012) examined habituation in a longitudinal study in which the same fetuses were examined at 35, 36, and 37 weeks of gestation. This study examined fetuses exposed to alcohol and considered factors of the amount of alcohol consumed by the mother and the way she consumed the alcohol (i.e., her drinking pattern). Fetuses were divided by their mother's drinking pattern into fetuses of mothers who drank as a binge, that is undertook their drinking in a confined period of 2–3 days/week or who were consistent drinkers, that is mothers who drank the same amount each day across the week. These groups were further subdivided by the amount of alcohol the mothers consumed into: heavy drinkers, mothers who consumed more than 20 units/week; and moderate drinkers, mothers who drank between 5 and 10 units/week. A control group of fetuses whose mothers never consumed alcohol during their pregnancy was also studied. Habituation was examined in response to presentations using the vibro-acoustic stimulator of 2-s duration with an inter-stimulus interval of 5 s.

The results of the study indicated that both the amount of alcohol consumed by the mother and her drinking pattern adversely influenced the habituation performance of her fetus. Fetuses took longer (i.e., required more stimulus presentations) to habituate if they had been exposed to alcohol compared to fetuses who had not been exposed to alcohol. Requiring more stimulus presentation to habituate is considered a sign of more inefficient habituation performance. Fetuses of mothers who drank heavily (an average of 22.5 units of alcohol/week) exhibited a greater decrement (required more stimulus presentations to cease responding) in habituation performance than did fetuses of mothers who drank moderately (an average of 7.5 units of alcohol/week). Similarly binge drinking by mothers resulted in their fetuses exhibiting a greater decrement in habituation performance than fetuses of mothers who drank consistently. Across all groups fetuses of mothers who drank in a binge and consumed large amounts of alcohol exhibited the greatest decrement in their habituation performance, whereas moderate drinkers who drank evenly across the week (approximately one drink per day) exhibited very little decrement in performance.

One aim of this study was to examine the consistency of brain function across time, in this case the 3 weeks of the study. Did the fetus' brain, as assessed by habituation performance, function the same each time the fetus was tested? By comparing the variability in individual habituation scores across the three test periods it was found that heavy binge drinking decreased the functional stability of the brain and introduced significantly more variability in performance than other conditions. Fetuses not exposed to alcohol habituated following the same number of stimulus presentations on each test whereas fetuses

exposed to a heavy binge pattern of alcohol consumption exhibited variability in the number of stimulus presentations required to habituate on each of the three tests.

Habituation can be regarded as a measure of information processing that involves functional integration between various parts of the brain, including the cortices to operate effectively (Hepper & Leader, 1996). This study found that alcohol decreased the efficiency of the brain to process information as evidenced through slower and more varied habituation performance. The results observed in the fetuses in this study are consistent with research undertaken on children who were exposed to alcohol as fetuses where deficits in learning, memory, perception and attention have been observed (Riley & McGee, 2005). The variability in habituation performance seen in fetuses exposed to alcohol is also consistent with observations in children following prenatal exposure to alcohol (e.g., in reaction times, Jacobson, Jacobson, & Sokol, 1994; Olsen, Feldman, Streissguth, Sampson, & Bookstein, 1998; Simmons, Thomas, Levy, & Riley, 2010). All mothers tested in this study had no alcohol in their bodies at the time of testing which may point to a likely chronic effect of exposure to alcohol. However, as discussed previously, the amount of alcohol that remains in the fetal body and for what duration is difficult to determine.

Exactly what areas of the brain are nvolved in the habituation response of the fetus, or even the adult, remain elusive. It is believed that functions such as novelty detection, orientation and attention, linked to the prefrontal cortex and hippocampus, are involved (Knight & Scabini, 1998; Lindsley, 1982). These are brain areas in which damage has been reported following exposure to alcohol in the third trimester (Guerri, Bazinet, & Riley, 2009). The observation of deficits in habituation observed in the fetus may represent the initial effects of structural damage to these areas as a result of alcohol exposure and underlie the deficits observed in psychological function in individuals after birth.

Observation of Fetal Behavior Advances Our Understanding of the Effects of Prenatal Alcohol Exposure

Research examining the effects of alcohol on the behavior of the human fetus provides insights into the actions of alcohol on the fetus and her brain and advances our understanding in a number of areas.

The Influence of Alcohol on the Behavior of the Fetus

One aim of this review was to demonstrate that observing the behavior of the fetus would provide insights into how exposure to alcohol affected brain function. The studies reviewed above elucidate some of the actions of alcohol on the behavior of the fetus. In summary, the behavior of the fetus is suppressed following acute exposure to alcohol. More chronic exposure induces a delay in the development of behavior, adversely affects the fetus' ability to process information, and decreases the stability of the fetus' brain functioning. The prenatal neurobehavioral effects induced by exposure to alcohol are exacerbated by greater alcohol consumption and by the pattern of maternal binge drinking, most likely a result of an increased intensity of alcohol exposure. However, the results also demonstrate that individual factors in both the mother and fetus influence the effect exerted by alcohol on the fetus.

The value of observing the behavior of the fetus to provide information on well-being, as illustrated through examination of the effects of alcohol exposure, is clear. The impact and longevity of exposure to acute alcohol exposure can be determined from the study of the behavior of the fetus. Possible mechanisms and sites of action can be elucidated by evaluating the effects of alcohol on behavior during the period of exposure. Importantly, the observed behavioral response reflects the actual effect of alcohol and is indepen-

dent of measures of exposure which may be incorrect or unobtainable, and may not correlate with outcome.

Thus observation of the behavior of the fetus provides a valuable tool in documenting the effects of alcohol on the fetus and his brain and provides opportunities for further understanding the effect of prenatal exposure to alcohol.

Insights into Mechanisms of Action

By studying the behavior of the fetus a greater understanding of the effects of alcohol on the fetus' brain and central nervous system has been obtained and this may enable an increased understanding of the underlying mechanisms of action. The observation of the fetus' behavior following maternal consumption of alcohol has revealed a number of intriguing results which may further our understanding of the effects of alcohol on the fetus.

Studies described above indicate that alcohol remains in the fetus' system for a considerable period of time and this raises the possibility of the prenatal acquisition of a preference for the flavor of alcohol. The human fetus learns about flavors experienced in the womb and shows a preference for them after birth (garlic, Hepper, 1995b; carrot, Mennella, Jagnow, & Beauchamp, 2001; anise, Schaal, Marlier, & Soussignan, 2000). Newborns, who as fetuses had experience of alcohol during gestation, exhibited a more pleasurable facial response to the odor of ethanol when presented to them than those newborns who had not been exposed to alcohol as fetuses during gestation (Faas, 2001). Recently, it has been demonstrated that prenatal exposure to garlic leads to a preference for garlic 8 years after birth (Hepper, Wells, Dornan, & Lynch, 2013), suggesting prenatal flavor exposure may exert a long-term effect on dietary preferences.

Observation of the spontaneous behavior of the fetus in response to acute exposure to alcohol reveals that movements are suppressed for 2–3 h following maternal consumption of a single glass of alcohol. It may be inferred that following a normal drinking pattern where more than a single drink is consumed and drinking is undertaken over a longer time period (hours) the period of behavioral suppression will last from the consumption of the first drink to a few hours after consumption of the last drink. Thus, the behavior of the fetus may be suppressed for a considerable period of time. Moreover, if the mother drinks on a daily basis then the suppression of movement will occur over a protracted period of time. Experiential effects contribute significantly to the development of the fetus (Hepper, 2015). The formation of normal joints and muscle tone requires their movement (Moessinger, 1988) and experience of certain sensory stimuli is important for the development of the brain (Blakemore & Cooper, 1970). If alcohol suppresses the fetus' normal movements and sensory experiences not only may physical development be affected but the development of neural pathways dependent on that stimulation also may be affected. Of particular note here is the suppression of fetal eye movements reported by Mulder et al. (1998). Rapid Eye Movement (REM) sleep is important for the development of the brain and the loss of this for long periods may adversely affect brain development (Visser et al., 2010). The effects of behavior being suppressed by alcohol exposure in the fetus have yet to be explored but observation of the behavior of the fetus has revealed a possible mechanism whereby some of the effects of alcohol exposure may be mediated.

One final observation to be highlighted here is the instability in brain performance observed as a result of alcohol exposure on habituation performance. A normal expectation is that our brain functions the same today as it did yesterday and will do so tomorrow. Early development is a crucial time for the acquisition of skills that provide the bedrock on which future development occurs. If the ability of the brain to perform consistently is hindered as a result of prenatal exposure to alcohol this may significantly influence the ability of an individual to acquire information as it develops and matures. The normal

path of acquiring information may be affected by differing performance of brain day-to-day and this may significantly influence the individual's development trajectory. Much more study is required to explore this issue.

Future Directions

The consistency between the observations of the effects of alcohol on the fetus and those undertaken on children/adults after birth suggests that the same fundamental adverse effects of exposure to alcohol are being identified at the time of exposure as are being identified after birth through studies of infants, children and adults. This is important as one potential value of using behavioral observations prenatally is the early identification of any adverse effects arising from exposure to alcohol. This may be particularly important given that diagnostic criteria for determining the presence of FASD have proved difficult to establish (Mattson & Riley, 2011). Of particular note are the case reports of abnormalities in state transition and eye movements following heavy exposure to alcohol (Mulder et al., 1986, 1998) which may, with further study, provide unique indicators of alcohol's effects on the fetus.

If one can identify the earliest indications of damage arising as a result of exposure to alcohol the possibilities for treatments and interventions are increased. This is particularly true of the prenatal period where the brain may be considered as being at its most plastic. Although no treatments to alleviate the damage caused to the brain by prenatal alcohol exposure are available at present, future years may see the development of treatments and their success will rely on the ability to identify deficits early.

Finally, one important area where additional information might be considered to be needed is in the advice provided to pregnant women about drinking during pregnancy. This is at best inconsistent, with differing statements regarding the necessity of abstinence and the "safe" amount of alcohol that can be consumed (BMA, 2007). Studies of the fetus have clearly indicated that a single glass of wine exerts an effect on the fetus, suppressing its behavior. This effect is immediate and may be long-lived depending upon exposure levels. Such observations clearly indicate that one glass of alcohol affects the fetus, and affects arguably its most important organ, its brain. As such this evidence should inform messages given to pregnant women, in particular, that even one drink affects the fetus.

Conclusion

In summary, observations of the behavior of the fetus have the potential to provide valuable information on fetal well-being and in particular the functioning and health of the fetus' brain. Arguably, observation of behavior provides the only method of meaningfully assessing fetal brain function and abnormalities in its performance, especially following exposure to environmental agents. More study should now be dedicated to exploring their value for monitoring health during perhaps the most important developmental stage of our lives.

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The Effects of Alcohol Exposure on Fetal Development

17

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Abstract

Fetal alcohol spectrum disorder (FASD) is the term used to describe the range of pervasive and long-lasting developmental, neurobehavioral, and physiological impairments induced by maternal alcohol (ethanol) consumption during pregnancy. Multiple factors interact to increase the risk of alcohol teratogenicity in the developing fetus, including the gestational timing, duration, and dose of alcohol exposure, amount consumed per drinking session, genetic and epigenetic factors, maternal and fetal stress, nutritional status, and alcohol metabolism capacity of the mother. Research findings from clinical and experimental animal models have characterized the range of fetal impairments produced by prenatal alcohol exposure (PAE), including defects in the developing brain, hypothalamic–pituitary–adrenal axis, heart, kidneys, lungs, auditory and visual systems, metabolic organs, and immune system. Importantly, PAE induces behavioral and cognitive deficits, which often represent the most pervasive and persistent manifestations of alcohol teratogenicity in offspring. The long-term effects of PAE may impact an individual's mental health, resulting in increased susceptibility to depression, anxiety, substance use disorders, and social behavior deficits throughout adolescence and adulthood. Results to date also suggest that PAE can increase the risk of cardiovascular disease, respiratory disorders, infection, inflammation, obesity, and diabetes in postnatal life. Currently, there is no identified safe level of maternal

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alcohol consumption during pregnancy. Therefore, it is advised that alcohol consumption should be avoided entirely throughout pregnancy to ensure optimal fetal development.

Keywords
Fetal alcohol spectrum disorder • Prenatal alcohol exposure • Fetus • Brain development • Alcohol teratogenicity • Organ systems

Introduction

Clinical and experimental studies have clearly demonstrated that alcohol (ethanol) consumption during pregnancy produces a range of pervasive and long-lasting developmental, neurobehavioral, and physiological impairments (Chudley et al., 2005; Sokol, Delaney-Black, & Nordstrom, 2003). The umbrella term, fetal alcohol spectrum disorder (FASD), is currently a non-diagnostic term used to describe the postnatal structural and functional defects produced by prenatal alcohol exposure (PAE), and encompasses four alcohol-related clinical diagnostic categories defined by the Institute of Medicine (Stratton, Howe, & Battaglia, 1996): fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (pFAS), alcohol-related neurodevelopmental disorder (ARND), and alcohol-related birth defects (ARBD) (Koren, Nulman, Chudley, & Loocke, 2003; Sokol et al., 2003). A diagnosis of FAS, with or without confirmed maternal ethanol consumption during pregnancy, requires the presence of three principal features: characteristic craniofacial dysmorphology, prenatal and postnatal growth restriction, and central nervous system (CNS) neurodevelopmental deficits (Jones, Smith, Ulleland, & Streissguth, 1973). Individuals that lack some of the characteristic facial features, but present with some of the other diagnostic criteria, may be diagnosed with pFAS if there is confirmed maternal ethanol consumption (Stratton et al., 1996). A diagnosis of ARBD requires confirmed maternal ethanol consumption and one or more congenital anomalies, including cardiac, skeletal, renal, ocular, and auditory malformations and dysplasias (Stratton et al., 1996). ARND is the most difficult diagnosis to make, since children present with normal growth and structural development, but display a complex pattern of behavioral and/or cognitive abnormalities induced by confirmed maternal alcohol consumption (Hoyme et al., 2005; Stratton et al., 1996). In addition to the formal diagnosis of an FASD, psychopathologies (e.g., anxiety, depression, other mood disorders, and substance use disorders), often referred to as "secondary disabilities," are expressed at disproportionally higher rates in individuals prenatally exposed to alcohol (Kodituwakku, 2007; O'Connor et al., 2002; O'Connor & Paley, 2006; Roebuck, Mattson, & Riley, 1999), which increases the health risks of these individuals as well as the emotional and financial cost to the family and health care system (Stade et al., 2009). Overall, the estimated incidence of FASD is thought to be approximately 1 in 100 live births, although recent epidemiological studies indicate prevalence rates as high as 2–5 % (May et al., 2009).

Risk Factors for Alcohol Teratogenicity

The potential impact of prenatal alcohol exposure varies considerably among exposed individuals, with some infants displaying serious alcohol-related effects and others showing no overt signs of an FASD (Khaole, Ramchandani, Viljoen, & Li, 2004). Therefore, it is important to understand the variables that may increase or decrease the probability that PAE will produce deleterious effects on fetal development. These factors include the gestational timing, duration, and dose of alcohol exposure (Astley, Magnuson, Omnell, & Clarren, 1999), amount consumed per drinking session (Khaole et al., 2004), genetic

and epigenetic factors (Kaminen-Ahola et al., 2010; Mantha, Laufer, & Singh, 2014), maternal and fetal stress (Weinberg, 1989; Weinberg & Bezio, 1987), nutritional status (Keen et al., 2010; Weinberg, 1985), and alcohol metabolism capacity of the mother, which are all known to be critically involved in alcohol teratogenicity (Ramchandani, Bosron, & Li, 2001; Riley, Infante, & Warren, 2011).

Dose, Duration, and Timing of Alcohol Exposure

Studies by Chernoff (1977), and Randall and Taylor (1979), verified a positive correlation between maternal blood alcohol concentration (BAC) and developmental malformations, a finding further validated by the seminal research of Pierce and West (1986), West, Goodlett, Bonthius, and Pierce (1989) and West, Kelly, and Pierce (1987). The use of varying alcohol treatment regimens in animal models has been critical in elucidating alcohol's teratogenic effects. Regimens may involve acute maternal administration (i.e., 1–2 doses) during critical periods of fetal development (Godin et al., 2010; Idrus & Napper, 2012; Sulik, Johnston, & Webb, 1981), trimester-equivalent fetal and/or neonatal alcohol exposure (Bake, Tingling, & Miranda, 2012; Hamilton, Boschen, Goodlett, Greenough, & Klintsova, 2012; Murawski, Jablonski, Brown, & Stanton, 2013; Wagner, Klintsova, Greenough, & Goodlett, 2013), intermittent binge-like exposure (Ramadoss, Wu, & Cudd, 2008; Sawant et al., 2013), or chronic exposure throughout gestation (Green et al., 2005; Hellemans, Verma, Yoon, Yu, & Weinberg, 2008; Iqbal et al., 2005; Iqbal, Brien, Kapoor, Matthews, & Reynolds, 2006; Probyn et al., 2013; Uban, Comeau, Ellis, Galea, & Weinberg, 2013).

Timing of alcohol exposure may be equally as important as BAC. In the early and mid-1980s, Webster, Walsh, Lipson, and Mcewen (1980), as well as Sulik and colleagues (Sulik & Johnston, 1983; Sulik & Schoenwolf, 1985), demonstrated that the timing of alcohol exposure was crucial to the development of craniofacial anomalies, a signature feature of FAS in humans. In addition, the timing of exposure can have major implications for brain development. The teratogenic effects of alcohol may be particularly detrimental to CNS development during critical periods of vulnerability, such as during the brain growth spurt, which occurs in the third trimester in the human and in the early postnatal period in the rat and mouse (Dobbing & Sands, 1979). Nevertheless, PAE can impair brain development throughout all stages of gestation, including effects on neurogenesis, differentiation, and synaptogenesis (Cudd, 2005; Dobbing & Sands, 1979; Guerri, 1998; West, Chen, & Pantazis, 1994). Indeed, using a rat model of PAE, early postnatal alcohol exposure has highlighted the enduring effects of PAE on neurogenesis, a process that continues throughout life in select areas of the brain, most notably the dentate gyrus within the hippocampus (Klintsova et al., 2007; Sliwowska et al., 2010; Uban et al., 2010).

Genetics, Maternal Nutrition, and Alcohol Metabolism Capacity

Various maternal factors act to increase the risk of alcohol teratogenicity in the developing fetus. Firstly, the presence of genetic polymorphisms of alcohol-metabolizing enzymes may significantly increase or decrease the risk of alcohol teratogenicity in the fetus. For example, maternal polymorphisms manifesting as increased alcohol dehydrogenase activity and enhanced alcohol metabolism have been associated with a decreased incidence of alcohol teratogenicity (Eriksson et al., 2001; Jacobson et al., 2006). Secondly, inadequate maternal nutrition may increase the risk of alcohol teratogenicity throughout fetal development. Deficiencies in various nutrients, including folic acid, iron, choline, and omega-3 fatty acids, are associated with alcohol-induced fetal growth restriction and neurobehavioral teratogenicity in postnatal offspring (Fuglestad et al., 2013; Idrus, Happer, & Thomas, 2013; Patten, Brocardo, & Christie, 2013; Rufer et al., 2012; Thomas, Idrus, Monk, & Dominguez, 2010; Wainwright et al., 1990; Wang et al., 2009).

Thirdly, the ability and extent of the maternal–fetal unit to metabolize alcohol may greatly affect the risk of alcohol teratogenicity. The capacity for alcohol metabolism among pregnant women varies up to eightfold (from 0.0025 to 0.02 g/dl/h), which may help to explain the variation in phenotypic presentation of FASD following maternal consumption of similar doses of alcohol (Burd, Blair, & Dropps, 2012). The capacity for alcohol metabolism in pregnant women varies with age, ethnicity, hormonal status, body composition and lean body mass, liver size, and food intake (Ramchandani et al., 2001). Ultimately, the peak BAC achieved from maternal alcohol consumption is critical to the risk of alcohol teratogenicity and depends on the rate of drinking, gastric emptying, and phase I biotransformation, involving alcohol dehydrogenase and cytochrome P450 2E1, as well as whether the mother is in a fed or a fasted state (Ramchandani et al., 2001). In general, however, mothers of children with FASD consumed more alcohol and achieved a higher BAC than mothers who consumed alcohol, but did not give birth to a child with FASD (Khaole et al., 2004).

Animal Models of Alcohol Teratogenicity

Early on, rodent models were key in demonstrating that BAC and timing of alcohol exposure are critical factors that affect the risk of alcohol teratogenicity, and in allowing for the control of specific experimental factors such as these. Numerous animal models have been utilized to this end, including drosophila (McClure, French, & Heberlein, 2011), zebrafish (Cole et al., 2012; McCarthy et al., 2013), chicken (Rao & Chaudhuri, 2007; Smith, 2008; Tan et al., 2013), mouse (Brady, Allan, & Caldwell, 2012a, 2012b; Gauthier, Ping, Gabelaia, & Brown, 2010), rat (Boehme et al., 2011; Hellemans et al., 2010b; Sliwowska et al., 2010; Thomas et al., 2010; Uban et al., 2013; Weinberg, 1989), guinea pig (Abdollah, Catlin, & Brien, 1993; Brien et al., 2006; Catlin, Abdollah, & Brien, 1993; Hewitt et al., 2011; Iqbal et al., 2006; Shea, Hewitt, Olmstead, Brien, & Reynolds, 2012), sheep (Goh et al., 2011; Gray, Denton, Cullen-McEwen, Bertram, & Moritz, 2010; Ramadoss, Wu, & Cudd, 2008; Sozo et al., 2011)), and nonhuman primate (Clarren et al., 1990; Creeley, Dikranian, Johnson, Farber, & Olney, 2013; Papia et al., 2010).

PAE can occur by injecting or intubating alcohol to the dam at specific times in gestation (Bake et al., 2012; Godin et al., 2010; Sulik et al., 1981), placing alcohol in the drinking water or in a liquid diet fed to the mother (Hewitt et al., 2010; Iqbal et al., 2005; Weinberg, 1989; Weinberg & Bezio, 1987) or feeding the offspring directly in the early postnatal period (third trimester equivalent) by intragastric (i.g.) intubation (West et al., 1987). However, due to stress often associated with many methods of alcohol administration, voluntary alcohol consumption represents a reliable and often preferred method of alcohol administration (Kleiber, Laufer, Wright, Diehl, & Singh, 2012; Weinberg, 1984). For example, the vervet monkey has recently been utilized in alcohol-related research owing to its voluntary and naturalistic pattern of alcohol consumption that results in moderate, but sustained BACs (Burke, Palmour, Ervin, & Ptito, 2009). Likewise, the miniature swine model has also proven to be useful, as it, too, consumes alcohol readily even when food and water are available (Dexter, Tumbleson, Hutcheson, & Middleton, 1976; Riley & Meyer, 1984). Indeed, because of its size and ease of handling, the miniature swine model has been particularly useful in studying the long-term effects of alcohol consumption, especially as they relate to alcohol exposure with successive pregnancies (Dexter, Tumbleson, Decker, & Middleton, 1980). Such research has added to the understanding that continued alcohol use intensifies the deleterious effects with each successive pregnancy, as determined by number of live births and litter size (Smith, Lancaster, Moss-Wells, Coles, & Falek, 1987).

Much of the current alcohol teratogenicity research using animal models is conducted in rodents, specifically rats and mice, due to the relatively low cost and short length of gestation of pregnant dams (Schneider, Moore, & Adkins,

2011). In addition, many of the clinically observed structural and functional features of FASD have been reproduced in rodent models of alcohol teratogenicity (Cudd, 2005). For instance, the facial dysmorphology of FAS—such as narrow forehead, short palpebral fissures, small nose, and thin upper lip with indistinct philtrum—is observed in fetal mice following alcohol administration by intraperitoneal injection on gestation day 7, which models binge alcohol exposure (Sulik et al., 1981). Moreover, rats and mice are well suited for performance in a wide range of neurobehavioral tasks, such as the Morris water maze, elevated plus maze, radial arm maze, T-maze, open-field, and sucrose preference tests (An & Zhang, 2013; Berman & Hannigan, 2000; Christie et al., 2005; Kim et al., 1997; O'Leary-Moore, McMechan, Mathison, Berman, & Hannigan, 2006; Ohta, Sakata-Haga, & Fukui, 2012; Sanchez Vega, Chong, & Burne, 2013; Thomas, Sather, & Whinery, 2008). However, there are some major considerations in using mouse or rat models to study the effects of PAE on fetal development. Most notably, the brain growth spurt, which is the period of highest velocity brain growth and development, extends into the early postnatal period in the rat and mouse from approximately postnatal day (PD) 1–10 (Dobbing & Sands, 1979). By contrast, in the human, the brain growth spurt (period of rapid neuronal growth and cell proliferation, synaptogenesis, dendritic arborization, and protein synthesis), a period highly sensitive to the effects of PAE, occurs during the third trimester of gestation (Cudd, 2005; Dobbing & Sands, 1979; Guerri, 1998; West et al., 1994). As such, some rodent studies initiate alcohol administration directly to the neonatal offspring after parturition as a "third trimester-equivalent" model (Cudd, 2005). While direct exposure of the neonate to alcohol has the advantage of controlling for the contributions of the placenta, maternal metabolism, and parturition to the effects of alcohol on the developing brain (Cudd, 2005), the disadvantage of this model is the lack of face validity. Indeed, it is likely that alcohol produces adverse outcomes in the developing fetus via direct and indirect (maternally mediated) targets (Randall, Ekblad, & Anton, 1990). Moreover, alcohol is known to act on or modulate different target molecules via multiple mechanisms, activated at different stages of development and at different dose thresholds of alcohol exposure, all of which likely contribute to the diverse phenotypes seen in FASD (Goodlett, Horn, & Zhou, 2005). For instance, alcohol may simultaneously and/or consecutively produce widespread cell death, disrupt axon migration, and impair neurochemical machinery that is necessary for normal development. As well, alcohol may disrupt development through endocrine or neuroendocrine imbalance and altered maternal–fetal hormonal interactions [for review see (Zhang, Sliwowska, & Weinberg, 2005)]. Thus, despite issues with generalization, the use of animal models of PAE continues to be of the utmost importance for informing outcomes and investigating mechanisms underlying the adverse effects of alcohol in human populations.

Effects of PAE on Fetal Development

Brain Development

Magnetic resonance imaging (MRI) studies have demonstrated that PAE causes widespread damage to the developing brain, resulting in structural injury to nearly every region (Lebel, Roussotte, & Sowell, 2011; O'Leary-Moore, Parnell, Lipinski, & Sulik, 2011), including the cerebellum (Paolozza, Titman, Brien, Munoz, & Reynolds, 2013; Sawant et al., 2013; Wagner et al., 2013), cerebral cortex (Bailey, Brien, & Reynolds, 2001; Dettmer et al., 2003; Puri, Reynolds, & Brien, 2003), the frontal and prefrontal cortex (Bailey et al., 2001; Green et al., 2009; Hamilton et al., 2010; Puri et al., 2003; Rasmussen, 2005), corpus callosum (Bookstein et al., 2007; Dodge et al., 2009; Livy & Elberger, 2008), and hippocampus (Abdollah et al., 1993; Astley et al., 2009; Barnes & Walker, 1981; Berman & Hannigan, 2000; Green et al., 2005; Hamilton et al., 2012; Iqbal, Brien et al., 2006; Joseph et al., 2014).

Alcohol-induced damage to any or all of these regions during fetal development can lead to persistent structural injury in adolescent and adult offspring (Lebel et al., 2011; O'Leary-Moore et al., 2011), with subsequent functional consequences. As noted, factors such as timing and duration of alcohol exposure, and BAC influence the severity of neurobehavioral dysfunction observed in individuals with FASD (Bailey, Brien, & Reynolds, 2004; Driscoll, Streissguth, & Riley, 1990). PAE-induced deficits in executive function (EF) and cognition are well documented and range from impairments in learning, including mathematical skills and comprehension (Kopera-Frye, Dehaene, & Streissguth, 1996; Streissguth et al., 1994a; Streissguth, Barr, Sampson, & Bookstein, 1994b), attention, and short-term memory (Streissguth et al., 1994), to disinhibition, impulsivity, and altered social behavior (Nash et al., 2006).

PAE induces morphologic and functional impairments in the developing CNS via a variety of mechanisms. Firstly, PAE impairs neurogenesis and neuronal cell proliferation, and results in mistimed or reduced neuronal and/or glial migration (Aronne, Guadagnoli, Fontanet, Evrard, & Brusco, 2011; Boehme et al., 2011; Gil-Mohapel et al., 2011; Klintsova et al., 2007; Liesi, 1997; Lindsley, Kerlin, & Rising, 2003; Ozer, Sarioglu, & Gure, 2000; Sliwowska et al., 2010; Uban et al., 2010). Alcohol also has been demonstrated to disrupt neurite outgrowth, synaptogenesis, and myelination (Aronne et al., 2011; Boehme et al., 2011; Gil-Mohapel et al., 2011; Klintsova et al., 2007; Liesi, 1997; Lindsley et al., 2003; Ozer et al., 2000; Sliwowska et al., 2010; Uban et al., 2010). Impaired function of neural cell adhesion molecules has been observed following PAE, which may alter cell-to-cell interactions in the developing brain (Bearer, Swick, O'Riordan, & Cheng, 1999; Gubitosi-Klug, Larimer, & Bearer, 2007; Minana et al., 2000). PAE also results in the formation of reactive oxygen species (ROS), leading to increased oxidative stress and decreased antioxidant capacity in the fetal brain, which may induce cell damage and apoptosis (Brocardo et al., 2012; Green et al., 2005, 2006; Kim et al., 2010; Ojeda, Nogales, Murillo, & Carreras, 2012; Patten et al., 2013; Patten, Brocardo, & Christie, 2013). Fetal brain development may also be disrupted by alcohol via alterations in glucose utilization and transport (Miller & Dow-Edwards, 1988; Snyder, Jiang, & Singh, 1992; Tan et al., 2013; Vorbrodt et al., 2001), altered gene expression (El Shawa, Abbott, & Huffman, 2013; Kleiber, Mantha, Stringer, & Singh, 2013; Mantha et al., 2014; Zink et al., 2011), epigenetic modifications and altered microRNA (miRNA) expression (Bekdash, Zhang, & Sarkar, 2013; Mantha et al., 2014; Stringer, Laufer, Kleiber, & Singh, 2013), and decreased DNA and protein synthesis (Canales, Gambrell, Chen, & Neal, 2013; Guerri & Renau-Piqueras, 1997; Shibley & Pennington, 1997).

Importantly, alcohol exposure during gestation also has been shown to impair neurotransmitter signaling, which is critical for fetal brain development and postnatal function. For example, PAE alters *N*-methyl-D-aspartate (NMDA) receptor expression and function, impairs glutamate and γ-aminobutyric acid (GABA) signaling, and modifies monoamine metabolism and receptor function (Brady et al., 2013; Honse, Nixon, Browning, & Leslie, 2003; Mao et al., 2013; Miller, 2006; Naassila & Daoust, 2002; Samudio-Ruiz, Allan, Valenzuela, Perrone-Bizzozero, & Caldwell, 2009; Sari, Hammad, Saleh, Rebec, & Mechref, 2010; Sickmann et al., 2014; Zhou, Wang, & Zhu, 2012). This alcohol-induced dysregulation of excitatory and inhibitory neurotransmitter systems may contribute to the increased incidence of seizure disorders in offspring (Bell et al., 2010; Riljak, Maresova, Jandova, Bortelova, & Pokorny, 2012). In addition, PAE may result in impaired insulin/insulin-like growth factor (IGF) signaling, leading to impaired cell proliferation, growth, viability, energy metabolism, synapse formation, and neurotransmitter production in the developing brain (De la Monte & Wands, 2010; Luo & Miller, 1998). In particular, alcohol exposure acts to down-regulate insulin/IGF signaling, resulting in various degrees of neuronal cell damage, including increased oxidative stress, DNA damage, lipid peroxidation, mitochondrial dysfunction, and reduced neuronal survival (Chu, Tong, & de la

Monte, 2007; De la Monte et al., 1999; De la Monte, Neely, Cannon, & Wands, 2001; De la Monte & Wands, 2002; Ramachandran et al., 2001; Zhang, Rubin, & Rooney, 1998). Furthermore, PAE may also disrupt various other cell signaling pathways that are critical for fetal brain development, including growth factor (Bonthius, Karacay, Dai, & Pantazis, 2003; Ceccanti et al., 2013; McCarthy et al., 2013; Miller, 2003), nitric oxide (Bonthius et al., 2003; Gibson, Butters, Reynolds, & Brien, 2000; Kimura, Reynolds, & Brien, 2000), and Wnt (Vangipuram & Lyman, 2012) signaling pathways. Important indirect mechanisms of alcohol teratogenicity include altered placental function, decreased umbilical artery blood flow, and changes in maternal–fetal hormone signaling, which may result in hypoxia and/or growth restriction of the fetus (Clave, Joya, Salat-Batlle, Garcia-Algar, & Vall, 2014; Cudd, Chen, Parnell, & West, 2001; Jones, Leichter, & Lee, 1981; Randall & Saulnier, 1995).

Neurobehavioral Consequences

Although the extent of the CNS impairment is generally associated with the BAC achieved by maternal alcohol consumption (Streissguth, Barr et al., 1994a), persistent cognitive impairments in the offspring may be observed at all levels of PAE (Driscoll et al., 1990; Green et al., 2009; Kodituwakku, 2009; Kodituwakku, May, Clericuzio, & Weers, 2001), making behavioral and cognitive deficits one of the most pervasive, persistent manifestations of FASD (Sampson et al., 1997; Streissguth & O'Malley, 2000). However, PAE-induced functional deficits vary considerably among individuals. For example, although not inevitable, PAE often results in one or a constellation of deficits that include reduced intelligence quotient (IQ), learning disabilities, hyperactivity, executive function deficits including attention problems and inappropriate behavioral responses, social behavior deficits, sleep disturbances, and psychiatric disorders (Chen, Olson, Picciano, Starr, & Owens, 2012; Green et al., 2009; Jacobson & Jacobson, 2002; Koren, Zelner, & Nash, 2014; Kully-Martens, Denys, Treit, Tamana, & Rasmussen, 2012; Mattson, Crocker, & Nguyen, 2011; Rasmussen, 2005; Riley et al., 2011; Steinhausen & Spohr, 1998).

Owing to the multitude of effects that PAE may have on developmental processes, the underlying mechanisms related to neurobehavioral and cognitive dysfunction are not yet well defined or understood. Moreover, as brain maturation is a dynamic process, the adverse effects of PAE during early gestation are potentially augmented during the ongoing process of development. The latter point is especially relevant to the development of executive function, as some of the regions that support these processes continue to mature into early adulthood (Anderson, Jacobs, & Anderson, 2008). Thus, although children as young as 6 years of age may show deficits in aspects of executive function (Fuglestad et al., 2014), specific characteristics of impaired executive function may not fully present until later in development. As an example, in a study of 8- to 16-year-olds with an FASD, Rasmussen and Bisanz (2009) reported that performance on verbal tests of executive function actually decreased with age. Interestingly, PAE-induced impairments in some cognitive processes are often observed only in complex tasks that require use of previously learned information, with sparing of function in simpler tasks (Streissguth et al., 1991).

The use of simpler brain systems to model complex human cognitive behavior has obvious limitations in elucidating mechanisms of alcohol-induced cognitive deficits in the developing organism. Nonetheless, many of the cognitive and behavioral impairments observed in individuals with FASD are paralleled in animal models, including deficits in memory, attention, inhibition, impulsivity, and social behavior (see Mihalick, Crandall, Langlois, Krienke, & Dube, 2001a for review). Importantly, many of the neurobiological mechanisms involved in aspects of cognitive behavior, such as learning and memory, are conserved across diverse species (Alkon, 1995). Thus, although one cannot model human cognitive processes as a whole, much can be garnered from animal models of FASD that focus on specific aspects of cognition, keeping in mind that cognition is relative to the organism under

study and that many neurobiological and molecular processes are conserved across species.

Numerous animal studies have demonstrated the effect of PAE on cognitive and neurobehavioral function in offspring. Following prenatal and/or early postnatal alcohol exposure, rat and mouse offspring have demonstrated impairments in spatial learning and memory tasks that are sensitive to hippocampal and/or prefrontal cortical injury, including impaired performance of T-maze (O'Leary-Moore et al., 2006; Ohta et al., 2012; Thomas et al., 2010; Wainwright et al., 1990; Zimmerberg, Sukel, & Stekler, 1991), Y-maze (Fernandez, Caul, Osborne, & Henderson, 1983; Kim et al., 2013; Osborne, Caul, & Fernandez, 1980), Morris water maze (An & Zhang, 2013; Christie et al., 2005; Endres et al., 2005; Girard, Xing, Ward, & Wainwright, 2000; Thomas et al., 2008, 2010; Wilcoxon, Kuo, Disterhoft, & Redei, 2005; Zink et al., 2011), and radial-arm maze (Brady et al., 2012a, 2012b; Neese, La Grange, Trujillo, & Romero, 2004; Omoto, Seki, Imai, & Nomura, 1993) tasks. In rats and mice, PAE offspring also have demonstrated hyperactivity in an open field, increased anxiety, attention deficits, increased response perseveration, and impaired social interactions compared with control offspring (Brocardo et al., 2012; Cullen, Burne, Lavidis, & Moritz, 2013a, 2013b; Hausknecht et al., 2005; Hellemans et al., 2008; Kim et al., 1997, 2013; Riley, Lochry, Shapiro, & Baldwin, 1979; Sanchez Vega et al., 2013; Staples, Rosenberg, Allen, Porch, & Savage, 2013). Consistent with these findings, PAE guinea pig offspring demonstrate hyperactivity in an open field (Abdollah et al., 1993; Bailey et al., 2001; Butters, Gibson, Reynolds, & Brien, 2000; Catlin et al., 1993; Gibson et al., 2000; Iqbal, Dringenberg, Brien, & Reynolds, 2004), impaired Morris water maze task performance (Iqbal et al., 2004; Iqbal, Rikhy, Dringenberg, Brien, & Reynolds, 2006; Nash, Ibram, Dringenberg, Reynolds, & Brien, 2007; Richardson, Byrnes, Brien, Reynolds, & Dringenberg, 2002), executive functioning deficits in a complex, multi-choice Biel maze (Dobson et al., 2012), and increased disinhibition and perseveration in neurobehavioral tasks (Hayward et al., 2004; Olmstead, Martin, Brien, & Reynolds, 2009).

HPA Axis Development and Fetal Programming Hypothesis

There is a growing appreciation for the impact that experience or environmental factors may have on prenatal and postnatal development and phenotypic outcomes. This area of study is especially pertinent to the individual with FASD, since maternal alcohol use is often associated with coinciding factors, such as poor nutrition, cigarette smoking, and illicit drug use (May & Gossage, 2011). Moreover, individuals with FASD are more likely to be raised outside of the birth family, with a high proportion entering into the foster care system, and many experiencing instability and other stressors early and/or throughout life (Stade et al., 2009). In addition, while not always the case, even those who remain in the family home may be at increased risk of early life adversity resulting from a chaotic and unstable environment, especially if the mother continues to consume alcohol and/or use other drugs (Streissguth & O'Malley, 2000). As such, prenatal and/or postnatal stress may become a mediating factor in the outcome of PAE.

The notion that stress may play an integral role in the effects of PAE is not novel. Indeed, alterations in the stress response system(s) may represent a potential mechanism by which alcohol or other early life exposures/experiences exert adverse effects during fetal development (for review, see Weinberg, Sliwowska, Lan, & Hellemans, 2008; Zhang et al., 2005). Data suggest that development of the hypothalamic–pituitary–adrenal (HPA) axis, an endocrine system involved in multiple metabolic functions and critical in the stress response, is altered or dysregulated by alcohol, likely through the mechanism of fetal programming.

The concept of fetal programming developed originally from studies showing that low birth weight was associated with increased risk for coronary heart disease, hypertension, and type 2 diabetes in adult life. These findings led to the "fetal origins of adult disease" hypothesis, the concept that common adult diseases might originate during fetal development (Barker et al., 1993). While the link between early growth restriction and long-term health consequences

are not fully understood, accumulating evidence suggests that the resetting of certain hormonal systems by early life events may be one mechanism underlying this link, and further, that the HPA axis may be one of the key systems involved. The HPA axis is highly susceptible to programming throughout development (Bakker, van Bel, & Heijnen, 2001; Kapoor & Matthews, 2005, 2008; Matthews, 2000, 2002; Matthews, Owen, Banjanin, & Andrews, 2002; Phillips et al., 1998, 2000; Phillips & Jones, 2006; Ward, Syddall, Wood, Chrousos, & Phillips, 2004; Welberg & Seckl, 2001) and strong correlations between birth weight, plasma cortisol concentrations, and the development of hypertension and Type II diabetes have been reported. Moreover, while optimal concentrations of glucocorticoid (GC) hormones are required for normal fetal brain development (Bohn, Bloom, Goldstein, & Black, 1984), and other developmental processes, including the maturation of fetal lungs, liver, thyroid, and gastrointestinal tract (Mesiano & Jaffe, 1997), excess concentrations of GCs during prenatal life are associated with suboptimal development, later HPA dysregulation, and increased risk for later life illnesses and psychopathologies (Mastorakos & Ilias, 2003).

Increased HPA activity following PAE has been reported in human infants (Jacobson, Bihun, & Chiodo, 1999; Ramsay, Bendersky, & Lewis, 1996), rats (Weinberg, 1989) and sheep (Cudd, Chen, & West, 2001; Ramadoss, Tress, Chen, & Cudd, 2008). Furthermore, data from animal models have shown that increased HPA reactivity to stressors persists into adolescence and adulthood (Lee, Imaki, Vale, & Rivier, 1990; Lee, Schmidt, Tilders, & Rivier, 2000; Redei, Clark, & McGivern, 1989; Taylor, Branch, Van Zuylen, & Redei, 1988; Weinberg et al., 2008).

The full impact of PAE-induced alterations in HPA axis activity on functional outcome in affected individuals is not fully understood. Of relevance, depression, anxiety disorders, and drug addiction all show high comorbidity with FASD, and are often referred to as secondary disabilities (Baer, Barr, Bookstein, Sampson, & Streissguth, 1998; Famy, Streissguth, & Unis, 1998; Kodituwakku, 2007; O'Connor et al., 2002; O'Connor & Kasari, 2000; O'Connor, O'Halloran, & Shanahan, 2000; O'Connor & Paley, 2006). In the context of fetal programming and the "fetal origins" hypothesis, one might hypothesize that adverse later life outcomes are not entirely secondary disabilities, but may be, at least in part, primary disorders, possibly mediated by fetal programming of HPA axis activity and regulation by alcohol exposure in utero.

Metabolic Consequences of PAE

Consistent with the "fetal origins" hypothesis, the mechanism of alcohol metabolic teratogenicity may be related to prenatal and/or early postnatal growth restriction and subsequent catch-up growth, which are associated with the development of symptoms of metabolic syndrome (Barker et al., 1993; Bertram & Hanson, 2001; Cettour-Rose et al., 2005; Hales & Barker, 1992). The thrifty phenotype hypothesis, first proposed by Barker and colleagues, describes the association between decreased birth weight and increased risk of several disorders in adulthood, including coronary heart disease, hypertension, stroke, and type 2 diabetes mellitus (Barker et al., 1993; De Boo & Harding, 2006; Hales & Barker, 1992). The hypothesis states that poor nutrition increases fetal metabolic efficiency and storage of fuel sources to protect critical tissues (e.g., brain) and enhance survival (Hales & Barker, 2001). However, the protection of brain tissue throughout fetal undernutrition occurs at the expense of peripheral tissues, such as skeletal muscle and the endocrine pancreas (Jones & Ozanne, 2009). The compensatory survival mechanisms observed in the fetus appear to persist into postnatal life. Consequently, as postnatal nutrient supply becomes adequate or excessive, offspring may demonstrate an increased risk of obesity and type 2 diabetes (Hales & Barker, 2001).

Several studies have demonstrated that PAE results in fetal growth restriction and subsequent catch-up growth, with adverse consequences for metabolic function both in fetal life and adulthood. For example, one study

demonstrated that PAE results in decreased body weight, reduced plasma insulin concentration, and increased plasma glucose concentration in newborn rat offspring (Chen & Nyomba, 2003a). Furthermore, at 13 weeks of age, these offspring demonstrated hyperglycemia and hyperinsulinemia after an intraperitoneal glucose tolerance test (GTT) (Chen & Nyomba, 2003a). As well, PAE resulted in decreased body weight in female rat fetuses, along with decreased serum insulin-like growth factor (IGF)-1 and triglyceride concentrations, increased serum glucose concentration, and alterations in liver and brain histology indicative of lipid accumulation (Shen et al., 2013; Xia et al., 2014), while PAE male offspring not only demonstrated growth restriction at birth but also a pattern of catch-up growth in postnatal life after exposure to a high-fat diet (Xia et al., 2014). Additionally, these offspring demonstrated insulin resistance, increased blood glucose and total cholesterol concentrations, and increased ratio of low-density lipoprotein (LDL) to high-density lipoprotein (HDL) in adulthood (Xia et al., 2014). A recent study indicated that the effect of PAE on metabolic function of offspring may extend to the next generation, as demonstrated by sex-dependent hypoglycemic and hyperinsulinemic GTT response patterns in F2 progeny (Harper, Tunc-Ozcan, Graf, & Redei, 2014).

The long-term effects of PAE on metabolic functioning may increase the risk of metabolic syndrome in offspring (Chen & Nyomba, 2003a, 2003b, 2004; Chen, Yao, & Nyomba, 2005; Chen, Zhang, & Nyomba, 2004; Elton, Pennington, Lynch, Carver, & Pennington, 2002; Fofana et al., 2010; Pennington, Shuvaeva, & Pennington, 2002a; Probyn, Parsonson et al., 2013; Shen et al., 2013; Tan et al., 2013; Villarroya & Mampel, 1985; Yao, Chen, & Nyomba, 2006; Yao, Nguyen, & Nyomba, 2013; Yao & Nyomba, 2008). In rodents, PAE impairs metabolic organ structure and function, including impaired glucose homeostasis, insulin resistance, increased gluconeogenesis, hypertriglyceridemia, hepatic lipid accumulation, and impaired insulin signaling, from early postnatal life through adulthood (Chen et al., 2004, 2005; Chen & Nyomba, 2003a, 2003b, 2004; Elton et al., 2002; Fofana et al., 2010; Pennington et al., 2002b; Probyn, Parsonson et al., 2013; Shen et al., 2013; Tan et al., 2013; Villarroya & Mampel, 1985; Yao et al., 2006, 2013; Yao & Nyomba, 2008). It has been suggested that lipotoxicity induced by PAE, involving increased lipid synthesis and lipid accumulation in non-adipose tissue (i.e., heart, liver, pancreas, skeletal muscle, and kidney), may play an important role in the development of adverse metabolic outcomes in offspring (Chen & Nyomba, 2004; Schaffer, 2003; Shen et al., 2013). PAE also has been shown to directly produce pancreatic damage and β-cell dysfunction, which may impair insulin production and/or secretion and result in glucose intolerance and insulin resistance in postnatal offspring (Cano, Ayala, Murillo, & Carreras, 2001; Cano, Garcia-Benitez, Ojeda, Murillo, & Carreras, 2007; Chen & Nyomba, 2003a). Indeed, several studies have demonstrated that PAE impairs insulin/IGF signaling in the liver and other peripheral tissues, resulting in long-lasting metabolic dysfunction. The effect of PAE on insulin/IGF signaling was amplified when female offspring were fed a high-fat diet, resulting in increased hepatic mRNA expression of IGF-1, IGF-1R, IRS-2, and GLUT2 (Shen et al., 2013).

Consistent with the data from animal models, a single small-scale study found that children with FAS were hyperinsulinemic and hyperglycemic in a GTT compared with typically developing children (Castells, Mark, Abaci, & Schwartz, 1981). More recently, 50 % of young females with PAE were found to be overweight or obese (Werts, Van Calcar, Wargowski, & Smith, 2013), whereas 37 % of males demonstrated reduced stature, body weight, or body mass index for their age (Werts et al., 2013). These results are supported by data demonstrating that young adult females with FASD showed increased adiposity, whereas growth deficiency was more common in young adult males (Spohr, Willms, & Steinhausen, 2007).

Alcohol can have direct and indirect effects on food consumption and metabolism in the pregnant

female, which can result in decreased fetal nutrition and fetal growth (Weinberg, 1985). A critical interaction between maternal undernutrition and alcohol consumption has been demonstrated, in which pregnant rats that underwent 30 % caloric restriction and alcohol treatment had decreased maternal IGF-1 mRNA and protein expression compared to undernourished controls (Shankar et al., 2006). Interestingly, fetal IGF-1 mRNA and protein expression was not affected by PAE alone or in combination with maternal undernutrition, suggesting that alcohol may selectively disrupt maternal growth factor signaling, resulting in reduced placental growth (Shankar et al., 2006).

As discussed above, developmental programming of the HPA axis by PAE may also represent a mechanism of alcohol metabolic teratogenicity, resulting in obesity, insulin resistance, and altered glucose metabolism in offspring (Chen & Nyomba, 2004; Hellemans et al., 2010a; McEwen, 2003; Rinne et al., 2002; Shen et al., 2013; Xia et al., 2014). A recent study proposed a "two-programming" hypothesis of alcohol metabolic teratogenicity, whereby prenatal programming of metabolic function (i.e., glucose homeostasis and lipid synthesis) is the first programming event, and postnatal adaptive catch-up growth triggered by the HPA axis and IGF acts as the second programming event (Shen et al., 2013). Indeed, a recent study found that a significant proportion (>50 %) of children with FASD did not achieve the Recommended Dietary Allowance (RDA) or Adequate Intake (AI) for numerous nutrients, including fiber, omega-3 fatty acids, vitamin D, vitamin E, vitamin K, choline, and calcium (Fuglestad et al., 2013). It is hypothesized that PAE increases the risk of nutritional deficiencies and inappropriate feeding behavior, which can result in sex-specific alteration in metabolic outcomes in offspring (Werts et al., 2013). Furthermore, it has been proposed that prenatal and postnatal dietary supplementation may mitigate many of the neurobehavioral effects of PAE in offspring (Idrus et al., 2013; Patten, Brocardo, & Christie, 2013; Rufer et al., 2012; Thomas et al., 2010; Wainwright et al., 1990; Wang et al., 2009).

Cardiovascular and Renal Development

Similar to the phenotypic variability in CNS injury in FASD, the occurrence of PAE-induced morphological and functional cardiovascular and renal defects is dependent on the timing and extent of PAE. Several animal studies, mostly utilizing a sheep model, have demonstrated the effect of PAE on fetal development of the cardiovascular and renal systems (Goh et al., 2011; Gray et al., 2008; Gray, Cullen-McEwen, Bertram, & Moritz, 2012; Moore, Khoury, & Liu, 1997; Morley et al., 2010; Ojeda et al., 2012; Probyn, Zanini, Ward, Bertram, & Moritz, 2012; Ren et al., 2002; Serrano, Han, Brinez, & Linask, 2010). A study by Goh et al. (2011) determined the effect of moderate daily alcohol administration in the pregnant sheep during late gestation, the period of cardiomyocyte maturation, on fetal cardiovascular development. The study demonstrated that heart-to-body ratio was increased in alcohol-exposed fetal sheep compared with controls, which was attributed to an increase in left ventricular and septal wall volume, an increase in cardiomyocyte size, and an increase in the proportion of mature, binucleated cardiomyocytes relative to immature, mononucleated cardiomyocytes (Goh et al., 2011). There was no effect of PAE on hemodynamics, as mean arterial pressure (MAP) in the fetus and postnatal offspring was unchanged compared with controls. The study concluded that PAE during late gestation accelerates the maturation of cardiomyocytes and induces left ventricular hypertrophy in the fetal heart, which may produce lasting effects on cardiovascular function. The study also demonstrated that PAE results in increased mRNA expression of pro-apoptotic genes, including caspase-3 and BAX, in the fetal sheep heart (Goh et al., 2011). Similarly, PAE resulted in enhanced caspase-3 activation and increased resting and peak intracellular calcium concentrations in the cardiomyocytes of rat offspring compared with control offspring (Ren et al., 2002). Furthermore, 10-week-old PAE rat offspring demonstrated altered myocardial contractile function compared with control offspring, suggesting

that PAE may result in an increased risk for cardiovascular disease in postnatal offspring (Ren et al., 2002). In a murine model, high-dose maternal alcohol administration (2.9–4.5 g/kg by intraperitoneal injection) at gestational day (GD) 6.75 resulted in abnormal echocardiogram patterns in embryos at GD 15.5, resulting in atrioventricular valve regurgitation and semilunar valve regurgitation (Serrano et al., 2010). PAE-induced cardiac defects also were observed in an avian model, including cardiac malformations and impaired cardiac gene expression (Serrano et al., 2010). Interestingly, folic acid supplementation prevented PAE-induced cardiac defects in both the murine and avian models. In a clinical study, 9-year-old children born to mothers who consumed alcohol during gestation demonstrated increased carotid-femoral pulse-wave velocity, a predictive marker of cardiovascular morbidity and mortality in adults (Morley et al., 2010).

Rodent and ovine models have demonstrated that PAE also results in impaired renal development. One study determined that moderate daily alcohol administration to pregnant sheep during late gestation resulted in an 11 % decrease in total nephron number in the near-term fetus, but did not affect kidney weight, glomerular volume, or mRNA expression of key regulators of renal development and function (Gray et al., 2008). It is hypothesized that the PAE-induced reduction in total nephron number is due to impaired renal branching morphogenesis, and that the observed decrease in fetal nephron number would persist into postnatal life (Gray et al., 2008). Similarly, acute alcohol administration to pregnant Sprague–Dawley rats at GD 13.5 and GD 14.5 resulted in a 10–15 % decrease in total nephron number at 1 month of age in male and female offspring (Gray et al., 2010). The same offspring demonstrated increased MAP and sex-dependent alterations in glomerular filtration rate (GFR) at 6 months of age (Gray et al., 2010). Likewise, low-to-moderate maternal alcohol consumption in pregnant Sprague–Dawley rats resulted in reduced kidney weight in male offspring at 8 months of age (Probyn et al., 2012). In addition, in vitro studies utilizing cultured rat kidney demonstrated that alcohol inhibits ureteric branching morphogenesis and glomerular development, which are both ameliorated by coculture with retinoic acid (Gray et al., 2012). Selenium and/or folate supplementation also mitigated PAE-induced renal damage in 21-day-old Wistar rat pups (Ojeda et al., 2012). An epidemiological study determined that there is an association between PAE and renal anomalies in offspring, including renal agenesis and hypoplasia, although larger-scale studies are required to confirm this finding (Moore et al., 1997).

Studies in animal models suggest that PAE results in significant impairments in cardiovascular and renal development, which may increase the risk of cardiovascular disease in later life. However, to date, there are no data on the incidence of cardiovascular disease in adolescents and adults with FASD, perhaps because the cohorts being studied, even the adolescents and adults, are still relatively young. Furthermore, future studies should determine whether risk factors, such as obesity, sedentary lifestyle, and/or poor diet, might result in greater risk for the development of cardiovascular disease in individuals with FASD who have impaired cardiac and renal function.

Auditory and Visual Systems Development

Alcohol exposure during fetal development results in various sensory deficits in offspring, which may play a major role in the neurodevelopmental impairments observed in FASD. One study determined that alcohol administration to pregnant Sprague–Dawley rats from GD 6 to GD 21 resulted in auditory brainstem response (ABR) abnormalities in offspring at PD 22 (Church et al., 2012). Interestingly, these auditory deficits largely dissipated in young adulthood (PD 220), but reappeared in later life (PD 520). The authors suggested that PAE delays normal development of auditory systems and increases susceptibility to age-related hearing loss in adulthood. Following an acute

maternal dose of alcohol at GD 12.5, mouse embryos exhibited increased apoptotic cell death in the vestibulocochlear ganglion complex, which may contribute to hearing and/or vestibular abnormalities in children with FASD (Du & Hamre, 2001). In fact, studies have shown that children with FAS demonstrate increased rates of mild to moderate sensorineural hearing loss, recurrent otitis media, and central auditory processing disorders (Church, Eldis, Blakley, & Bawle, 1997; Church & Gerkin, 1988).

Studies in various animal models, including the zebrafish, mouse, and chick embryo, have determined that visual system development also is impaired by PAE, resulting in ocular malformations and impaired visual function (Bilotta, Saszik, Givin, Hardesty, & Sutherland, 2002; Parnell et al., 2006; Tufan, Abban, Akdogan, Erdogan, & Ozogul, 2007; Zhang, Turton, Mackinnon, Sulik, & Cole, 2011). In the mouse, maternal alcohol exposure on GD 7 and GD 8 resulted in ocular malformations in >25 % of fetuses at GD 14. PAE also resulted in dose-dependent retinal degeneration, optic nerve hypoplasia, and reduced myelination of nerve fibers in a chick embryo model system (Tufan et al., 2007). Clinical studies have shown that PAE results in various congenital abnormalities affecting the eyes, including optic nerve hypoplasia and micropthalmia (Stromland, 2004).

Pulmonary Development

Recent evidence suggests that PAE impairs fetal lung development, which may result in long-lasting respiratory disorders, including increased susceptibility to infections (Johnson, Knight, Marmer, & Steele, 1981). For example, a study utilizing a mouse model of PAE throughout gestation determined that interstitial and alveolar macrophage phagocytic function and differentiation were impaired in the lungs of pups at term, which was attributed to increased oxidative stress and elevated transforming growth factor beta-1 (TGF-β1) concentration (Gauthier et al., 2010). Similar results were observed in sheep, in which moderate daily maternal alcohol administration during late gestation resulted in altered innate immune status, increased collagen deposition, and decreased surfactant protein mRNA expression in the fetal lungs (Sozo et al., 2009). However, studies in 9-week-old lamb offspring determined that the effects of PAE on pulmonary development did not appear to be persistent, as there was no difference in lung weight, collagen content, proinflammatory cytokine mRNA expression, or surfactant phospholipid composition (Sozo et al., 2011). Furthermore, although fetal sheep demonstrated reduced mRNA expression of various markers of lung maturation and immunity, including vascular endothelial growth factor-A (VEGF-A) and its receptor, hypoxia-inducible factor-1α (HIF-1α), HIF-2α, TNF-α, and IL-10, following third-trimester-equivalent PAE (Lazic et al., 2011), impairments in markers of fetal lung maturation did not appear to persist in full-term animals. In contrast, the immune alterations in PAE fetal sheep were persistent, suggesting that PAE may increase the risk of respiratory infections in offspring. A study by Wang, Gomutputra, Wolgemuth, and Baxi (2007) in the rat also found that high-dose PAE on GD 11.5, 12.5, and 13.5 resulted in decreased fetal lung weight and aberrant lung morphology at GD 18. Furthermore, a recent study showed that low-to-moderate PAE throughout gestation in Sprague–Dawley rats results in structural and functional alterations in the fetal and postnatal lung (Probyn, Cuffe, Zanini, & Moritz, 2013). In particular, male PAE offspring demonstrated in the lung increased collagen type III α1 mRNA expression at 8 months, increased collagen deposition at 10 months, and decreased surfactant protein B content at 19 months of age (Probyn, Cuffe et al., 2013, 2013, Probyn, Parsonson et al., 2013).

Further research is needed to understand the functional implications of altered pulmonary development in FASD. Possible effects of altered pulmonary system function on the heart, vascular development, and other organ systems, and the role of altered lung function in immune and inflammatory disorders described in FASD remain to be fully elucidated.

Immune System Development

There has been extensive interest in investigating the effects of PAE on the development and function of the immune system. A review of immune deficits in infants and children with FASD showed a higher incidence of minor infections, such as recurrent otitis media and respiratory infections, as well as major life-threatening bacterial infections, in those with FASD compared to non-alcohol-exposed children (Johnson et al., 1981). Similarly, work in animal models of PAE has reported an adverse impact of alcohol on numerous immune parameters, including white blood cell counts and responses to mitogens (reviewed in Abel & Hannigan, 1995; Dietert & Piepenbrink, 2008; Zhang et al., 2005).

Recent studies have extended these initial findings on the adverse effects of PAE, demonstrating inborn errors of immunocompetent cells, which could result in immunodeficiency disorders and/or increased susceptibility to infections (see review by Bodnar & Weinberg, 2013). The finding of an increased risk for recurrent otitis media, first reported by Johnson et al. (1981), was extended by studies suggesting that such recurring infections may be a risk factor for subsequent hearing loss and ensuing speech and language problems, as well as learning disorders that are common in children with FAS (Church et al., 1997). Data also have demonstrated that alcohol-exposed very low birth weight infants have a 15-fold higher incidence of early-onset sepsis (presence of microorganisms in blood cultures within 72 h of birth) compared to a matched control group (Gauthier, Manar, & Brown, 2004). Importantly, the level of alcohol intake by the pregnant female can be used to predict neonatal infection risk (Gauthier, Drews-Botsch, Falek, Coles, & Brown, 2005). In addition, studies have reported an increased incidence of malignancies following PAE, particularly those of embryonic origin (Becker, Zaunschirm, Muntean, & Domej, 1982), as well as a link between maternal alcohol consumption and increased risk of childhood leukemia (Latino-Martel et al., 2010; Shu et al., 1996; Van Duijn, van Steensel-Moll, Coebergh, & van Zanen, 1994).

Studies involving rodent models have both confirmed and extended the initial clinical findings of alterations in immune competence following PAE (reviewed in Zhang et al., 2005). An increased incidence of opportunistic bacterial infections, increased susceptibility to infections, alterations in lymphoid organ development, decreased T-lymphocyte responses to mitogen stimulation, and long-term deficits in adaptive immunity, all have been shown to occur following PAE (Becker, Diaz-Granados, & Randall, 1996; Ewald & Frost, 1987; Gottesfeld & LeGrue, 1990; McGill et al., 2009; Zhang et al., 2005). Immune organs, including the thymus, were shown to be altered by PAE, with studies reporting decreased thymus weight, size, cell number and mitogen-induced thymocyte proliferation (Ewald & Frost, 1987; Ewald & Walden, 1988; Redei et al., 1989). Finally, studies conducted examining the effects of paternal alcohol exposure, either in combination with maternal alcohol exposure (Hazlett, Barrett, Berk, & Abel, 1989) or alone (Berk, Montgomery, Hazlett, & Abel, 1989), have shown that paternal alcohol exposure for a 7-week period results in increased severity of ocular infection in adult offspring (Berk et al., 1989).

Importantly, many of the PAE-induced alterations in immune competence are shown to persist into adolescence and adulthood, with obvious health consequences. The mechanism(s) by which these PAE-induced alterations in immune cell populations, organs, and response to challenge are maintained across the life span, particularly with the high rate of turnover of most immune cell populations, remains to be determined.

Recent research has aimed at understanding the mechanisms underlying the altered immune function following PAE. It has been suggested that chronic alcohol consumption during pregnancy, a time during which Th2 proinflammatory responses are minimized to protect the fetus, may increase concentrations of pro-inflammatory cytokines, which may be important in alcohol's adverse effects on the fetus. For example, several lines of evidence link alterations in fetal cytokine concentrations to alterations in brain development, cytokine overproduction, and increased sensitivity to

later-life infections (Meyer, Feldon, & Yee, 2009; Pang, Cai, & Rhodes, 2003). However, many of these findings stem from maternal immune challenge models. Further research will be required to elucidate the nature, timing, and mechanism of cytokine transfer between mother and fetus in order to understand the possible role of proinflammatory cytokines in alcohol's adverse effects on offspring immune function.

PAE has been shown to impact the developmental trajectory of microglial cells in the brain (Kane et al., 2011). Microglia are resident macrophages of the CNS. They arise early during development (by GD 14 in the rodent) and exist initially in an activated state, producing cytokines, which are critical for brain development (Fujita, Tsuchihashi, & Kitamura, 1981). By postnatal day (PD) 6, these cells start to transition into a quiescent, ramified state and become inactive (Ling & Wong, 1993). As microglial cells are long-lived, it has been hypothesized that early-life exposure to toxic agents, such as alcohol, may impact microglial development and activity. In support of this hypothesis, studies have shown that alcohol exposure in vitro compromises microglial survival, affects morphology, and decreases microglial cell population of the cerebellum following neonatal ("third-trimester-equivalent") exposure (Kane et al., 2011). If microglial development is altered by alcohol, this could result in microglial "priming" during development, making these cells more easily stimulated in adulthood. Thus, in turn, this could result in the elicitation of inappropriate microglial responses following later-life immune challenges. Again, further studies will be required in order to fully understand alcohol's adverse effects on microglia, and how these effects may contribute to altered immune competence of offspring.

Effects of PAE on Mental Health in Adolescence and Adulthood

In addition to primary developmental deficits that are direct consequences of the teratogenicity of alcohol, a number of so-called secondary disabilities, such as impairment in reciprocal social interactions, deficits in metacognition, impaired judgment, and mental health problems, including depression, anxiety, and substance-use disorders, may develop in individuals with PAE (Banakar, Kudlur, & George, 2009; Famy et al., 1998; O'Connor et al., 2002; O'Connor & Paley, 2009). Moreover, given the known overlap of neurocircuitry for depression/anxiety, addiction, and HPA regulation, it is possible that dysregulation of HPA axis induced by PAE may underlie increased susceptibility to the onset of mental health problems in individuals with FASD (reviewed in Maniam, Antoniadis, & Morris, 2014).

Depression, Anxiety and Substance Use Disorders

Much of our current understanding of FASD and depression has come from rodent models, which have been instrumental in replicating the consequence of PAE on depression-/anxiety-related changes in both the physiology and behavior of the offspring. For example, maternal alcohol consumption has been shown to result in alterations in monoamine systems (Sari & Zhou, 2004; Zhou, Sari, & Powrozek, 2005; Zhou, Sari, Zhang, Goodlett, & Li, 2001), reduced brain BDNF concentration similar to that observed in postmortem brains of humans with depression (Caldwell et al., 2008), and increased depressive-like behaviors, including increased learned helplessness and immobility in the Porsolt forced swim task (FST) (Caldwell et al., 2008).

Alcohol exposure during various phases of prenatal and postnatal development result in offspring that present abnormalities in monoamine systems similar to those of depressed human patients (Blanchard et al., 1993; Druse, Kuo, & Tajuddin, 1991; Druse, Tajuddin, Kuo, & Connerty, 1990; Rathbun & Druse, 1985; Sliwowska et al., 2008; Zafar, Shelat, Redei, & Tejani-Butt, 2000). Rat models also have been used to study the interaction between the HPA axis and the serotonin system in depression. Offspring prenatally exposed to alcohol were found to exhibit increased serotonin 5-HT_{1A}

receptor-mediated anxiety-like behavior in adulthood (Hofmann, Patyk, & Weinberg, 2005), as well as altered interactions between the HPA and 5-HT systems (Hofmann, Ellis, Yu, & Weinberg, 2007). Moreover, the HPA changes induced by PAE include HPA hyperactivity in response to stressors (reviewed by Weinberg et al., 2008), increased HPA drive (Gabriel, Glavas, Ellis, & Weinberg, 2005; Glavas, Ellis, Yu, & Weinberg, 2007; Glavas, Hofmann, Yu, & Weinberg, 2001; Lee et al., 1990, 2000; Redei, Halasz, Li, Prystowsky, & Aird, 1993), deficits in HPA feedback regulation (Glavas, Ellis, Yu, & Weinberg, 2000; Hofmann, Glavas, Yu, & Weinberg, 1999; Osborn, Kim, Yu, Herbert, & Weinberg, 1996), downregulation of pituitary CRH type 1 receptor mRNA (Glavas et al., 2007), and altered responses to the dexamethasone (DEX) suppression test and the DEX-CRH test (Osborn et al., 1996; Osborn, Yu, Stelzl, & Weinberg, 2000), all of which parallel in many respects the changes seen with depression (Board, Wadeson, & Persky, 1957; Carroll et al., 1981; Deuschle et al., 1997; Yehuda, Teicher, Trestman, Levengood, & Siever, 1996). Importantly, alterations in HPA activity and regulation are found in children with FASD, as noted above (Haley, Handmaker, & Lowe, 2006; Jacobson et al., 1999; Ramsay et al., 1996). Together, these findings provide support for the possibility that fetal programming of the HPA axis by PAE may underlie, in part, the increased vulnerability to depression and anxiety in individuals with FASD.

Like depression/anxiety disorders, many individuals with FASD develop substance use/addiction problems (O'Connor & Paley, 2009). Indeed, PAE has been shown to be a better predictor of alcohol abuse in postnatal life than family history of alcohol abuse, or prenatal exposure to other substances (Alati et al., 2006; Baer et al., 1998; Baer, Sampson, Barr, Connor, & Streissguth, 2003). The mechanism by which PAE increases the propensity for addiction in later life is not known. However, alterations in developing neurobiological systems involved in reinforcement/motivation likely underlie, at least in part, the enhanced risk for addiction in this population. The influence of these alterations is likely further influenced by genetic, social, neurobiological, and environmental factors (Baer et al., 2003; Derijk & de Kloet, 2008; Li et al., 2001). Moreover, neurocircuitry implicated in reinforcement/motivation overlaps and interacts with stress system neurocircuitry, and this interaction likely plays an important role in risk for or resilience against development of addiction.

Rodent studies have shown that repeated PAE (GD 17–20) increases alcohol consumption and alcohol-related learning in offspring. Even a single day of PAE (GD 8) produces increased alcohol consumption in offspring (Molina, Hoffmann, Spear, & Spear, 1987), and additional postnatal alcohol exposure may enhance this effect (Holloway & Tapp, 1978). Enhanced alcohol preference also has been demonstrated using the conditioned place-preference (CPP) paradigm (Barbier et al., 2009). Similarly, enhanced preference for alcohol odor was found after an acute administration of alcohol into the amniotic fluid (GD 21) and after repeated maternal intragastric intubation (2 g/kg) from GD 17–20 (Abate, Varlinskaya, Cheslock, Spear, & Molina, 2002; Chotro, Cordoba, & Molina, 1991). PAE reduced sensitivity to alcohol-induced sedation (assessed using "loss of righting reflex"), which was associated with enhanced preference for and consumption of alcohol (Barbier et al., 2009). Of note, PAE also has been shown to alter responses to drugs other than alcohol, including psychostimulants such as cocaine and *d*-amphetamine (Barbier et al., 2009; Blanchard, Hannigan, & Riley, 1987).

PAE rodent models have been important in demonstrating altered function of key neurotransmitters important in reinforcement and motivation. For example, PAE alters development of dopaminergic systems (Choong & Shen, 2004a, 2004b; Kaneko, Riley, & Ehlers, 1993; Maciag et al., 2006; Shen & Choong, 2006; Shen, Hannigan, & Kapatos, 1999; Shetty, Burrows, & Phillips, 1993; Xu & Shen, 2001), alters sensitivity of presynaptic and postsynaptic dopamine receptors (Hannigan, 1996; Shen et al., 1999), and appears to result in hyper-dopaminergic function (Blanchard et al., 1993; Hannigan, 1990; Hannigan, Blanchard, Horner, Riley, & Pilati, 1990; Nowak et al., 2006). As well, PAE alters

development and activity of opioid and glutamatergic systems, which are important in understanding the mechanisms underlying addiction (Arias & Chotro, 2005). Furthermore, interactive effects of the HPA hormones and stress with neurotransmitter systems and the neurocircuitry of addiction have been reported for all addictive substances (Koob, 2008; Koob & Kreek, 2007; Koob & Le Moal, 2008), and understanding this reciprocal interaction has been helpful in elucidating individual differences in risk for developing addiction (Uban et al., 2009; Uban, Poursoltani, Comeau, Galea, & Weinberg, 2010). Of particular relevance to this review, recent studies suggest that PAE alters the cross talk between stress and dopaminergic systems (Nelson, Lewis, Liebeskind, Branch, & Taylor, 1983; Taylor, Branch, Nelson, Lane, & Poland, 1986) and may do so in a manner consistent with increased neurobiological sensitivity of underlying dopamine and stress systems to drug exposure [Uban, Poursoltani et al., 2010; Uban et al., 2009; Uban et al. (under review)]. Understanding the recruitment of stress pathways by drug exposure may provide valuable insight into the effects of PAE on risk of addiction, as well as potentially sensitive prenatal periods during development of stress and dopamine systems. However, further studies are needed to understand fully the mechanisms underlying this effect.

Social Behavior Deficits

Social behavior deficits following PAE have been characterized early in development, with infants showing disorganized attachment to caregivers that may be exacerbated by disrupted sleep/feeding cycles (O'Connor, Sigman, & Kasari, 2008; Platzman, Coles, Lynch, Bard, & Brown, 2001). Social deficits appear to persist and worsen with the transition into adolescence (Thomas, Kelly, Mattson, & Riley, 1998; Whaley, O'Connor, & Gunderson, 2001). Moreover, these individuals show increased rates of impulsivity and distractibility, which can further contribute to poor social functioning (Olson et al., 1997; Olson, Oti, Gelo, & Beck, 2009; Olson, Sampson, Barr, Streissguth, & Bookstein, 1992). Social behavior deficits are not merely a secondary effect of cognitive deficits or intellectual dysfunction, as children with FASD scored lower on general social skills when compared to children not exposed to alcohol but with similar verbal IQs (Mattson & Riley, 2000; Thomas et al., 1998). Moreover, individuals with FASD continue to exhibit deficits in social responsiveness and interpersonal relationships into adulthood (Kelly, Day, & Streissguth, 2000b; Kully-Martens et al., 2012), and consistently lag behind peers in social behavior function (Streissguth et al., 1991).

Animal models of PAE have shown social behavior deficits parallel to those observed in individuals with FASD (Hamilton et al., 2010a; Hellemans et al., 2010c; Kelly et al., 2000a; Kelly, Goodlett, & Hannigan, 2009). In neonatal rats, PAE has been linked with disrupted attachment (Subramanian, 1992) and reduced ability to elicit retrieval by the mother (Marino, Cronise, Lugo, & Kelly, 2002; Ness & Franchina, 1990). PAE-related social deficits persist into adolescence, including disruptions in play behavior (Lawrence, Bonner, Newsom, & Kelly, 2008) and deficits in social interaction (Middleton, Varlinskaya, & Mooney, 2012; Mooney & Varlinskaya, 2011) in adulthood, including increases in aggression (Royalty, 1990) and aberrant social interactions (Hamilton, Akers et al., 2010b). Adult PAE rats show sexually dimorphic patterns of altered social interactions with age- and sex-matched rats, with males showing reductions in the time spent in both affiliative and non-affiliative behaviors, and PAE females showing no change in the frequency of non-affiliative behaviors compared to their control counterparts (Hellemans et al., 2010c).

Social behavior's underlying neurobiology involves the complex interplay of many neural structures and neuroendocrine systems (Bielsky & Young, 2004; Ferguson, Young, & Insel, 2002; Ross & Young, 2009; Veenema & Neumann, 2008). Among these, the amygdala (Amaral, 2003; Katayama et al., 2009), prefrontal cortex (PFC; Bell, McCaffrey, Forgie, Kolb, & Pellis, 2009; Diamond, 2011), and hypothalamus (Ross & Young, 2009) are key areas that modulate fundamental aspects of socio-emotional processing in human and nonhuman primates and rats

(Amaral, 2003; Emery et al., 2001; Thomas et al., 2001). Interestingly, PAE produces sexually dimorphic structural and functional alterations in the amygdala (Cullen et al., 2013a, 2013b; Kelly & Dillingham, 1994; Zhou, Wang, & Zhu, 2010) and PFC (Hamilton, Akers et al., 2010a; Lawrence, Otero, & Kelly, 2012; Mihalick et al., 2001b), suggesting that these alterations may, at least in part, underlie social behavior deficits observed in individuals with FASD.

Oxytocin and vasopressin are key neuropeptides involved in regulating social behavior (Ostrowski, 1998; Ostrowski, Lolait, & Young, 1994) and have been implicated in mediating a variety of other functions, including the stress response (Onaka, Takayanagi, & Yoshida, 2012). Interestingly, Kelly, Leggett, and Cronise (2009) demonstrated that PAE results in sexually dimorphic deficits in social recognition memory and reductions in oxytocin-receptor binding in the amygdala of PAE female, but not male, adult rats. Moreover, PAE has been shown to reduce oxytocin expression within the hypothalamus in mandarin voles, specifically within the paraventricular and supraoptic nuclei (He, Zhang, & Guo, 2012). Future studies will be important in characterizing the possible role of oxytocin and vasopressin in the social behavior deficits seen following PAE. Such studies will have important implications for the development of treatments to attenuate social behavior deficits in FASD.

Conclusions

Extensive research in humans and animal models has demonstrated that PAE produces a plethora of adverse effects in the developing fetus. Alcohol exposure significantly impairs development of the fetal brain, HPA axis, heart, kidneys, lungs, auditory and visual systems, metabolic organs, and immune system, as well as other organ systems. The effects of PAE are long-lasting and often persist into adolescence and adulthood. Although further research is required to validate the existing data, results to date suggest that PAE can result in increased susceptibility to or risk for mental illness, social behavior deficits and addiction in postnatal life, as well as cardiovascular disease, respiratory disorders, infection, inflammation, obesity and diabetes. Studies utilizing various models of PAE, ranging from maternal binge-like administration to low-dose volitional consumption, have demonstrated that alcohol can have long-term adverse effects. At this time, there is no identified safe level of maternal alcohol consumption, and therefore, the best advice is that alcohol consumption should be avoided entirely during pregnancy to ensure optimal fetal development.

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18 Fetal Effects of In Utero Serotonin Reuptake Inhibitor (SRI) Antidepressant Exposure

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Abstract

In utero serotonin reuptake inhibitor (SRI) exposure is relatively common and neonatal behavioral outcomes vary greatly. Such exposure has led to questions about whether these effects reflect an acute short-lived pharmacological phenomenon that results in a "withdrawal" condition, or sustained neurological changes associated with altered serotonin (5-HT) signaling that begins long before birth. Emerging reports now suggest that certain effects associated with in utero SRI exposure become evident during gestation. For instance, in utero SRI exposure appears to influence fetal brain blood flow and neurobehavior. In this chapter, we summarize current research evidence reporting the fetal effects of in utero SRI exposure. Given the paucity of empiric fetal data in humans, we draw from what we know about three postnatal findings that may reflect fetal developmental sequelae associated with in utero SRI exposure.

Keywords

Antidepressants • Pharmacodynamics • Pregnancy • Depression • Fetus

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Introduction

Serotonin reuptake inhibitor (SRI) antidepressants are the most commonly used psychotropic medicine in pregnancy (Hanley & Mintzes, 2014). Between 15 and 20 % of women experience mood disorders (e.g., depression) during their pregnancy and 5–13 % of women with an antenatal mood disorder will be treated with an SRI antidepressant (Cooper, Willy, Pont, & Ray, 2007; Hanley & Mintzes, 2014; Oberlander, Warburton, Misri, Aghajanian, & Hertzman, 2006). Soon after the introduction of SRIs in the late 1980s, reports of preterm birth, low birth weight, pulmonary hypertension, and a cluster of neonatal "withdrawal" symptoms appeared, suggesting gestational and neurobehavioral disturbances associated with in utero SRI exposure (Oberlander et al., 2002). Importantly, some (Croen, Grether, Yoshida, Odouli, & Hendrick, 2011; Zeskind & Stephens, 2004), but not all (Nulman et al., 1997; Pedersen, Henriksen, Vestergaard, Olsen, & Bech, 2009; Stephansson et al., 2013) studies reported neurobehavioral disturbances, leaving critical unanswered questions about whether fetal SRI exposure-related outcomes reflect a transient pharmacological effect, suppressed neurotransmitters, or sustained alterations in brain development that starts long before birth.

The management of antenatal maternal mood disturbances presents an important public health concern as attempting to minimize risk in the fetus while optimizing maternal benefits makes choice of treatment uniquely challenging. Up to 50 % of women discontinue their medication within the first 60 days of their pregnancy (Bennett, Einarson, Taddio, Koren, & Einarson, 2004; Oberlander et al., 2006; Vesga-López et al., 2008; Warburton, Hertzman, & Oberlander, 2010), highlighting the urgency to recognize and manage perinatal mood disturbances and to establish evidence to guide antenatal SRI use in conjunction with non-pharmacological options (Yonkers et al., 2009). Thus, it is critical to understand the pharmacological and physiological effects when weighing the risks and benefits of SRI medication use in pregnant women—particularly in relation to maternal mental health and infant neurodevelopment (Yonkers et al., 2009).

Emerging findings point to evidence of biological and behavioral effects long before symptoms appear after delivery. While some biobehavioral effects associated with in utero SRI exposure are apparent during fetal periods, other effects emerge in the newborn period and over the first year of life. Given the paucity of fetal studies that provide direct evidence of fetal disturbances, this chapter draws from outcome data across infancy and early childhood to illustrate the potential impact of in utero SRI exposure on fetal development.

The Role of Serotonin

Serotonin (5-HT) is a phylogenetically ancient neurotransmitter widely distributed throughout the brain and already functional by mid-gestation (Kalueff, Olivier, Nonkes, & Homberg, 2010; Lebrand, Gaspar, Nicolas, & Hornung, 2006). Serotonin not only acts as a neurotransmitter in the mature brain regulating mood, appetite, and sleep, but also plays a neurodevelopmental role as a trophic signal long before birth, regulating the development of its own and related neural systems (Whitaker-Azmitia, Druse, Walker, & Lauder, 1996). In this way, 5-HT regulates diverse and developmentally critical processes in the fetal brain such as cell division, differentiation, migration, myelination, synaptogenesis, and dendritic pruning (Gaspar, Cases, & Maroteaux, 2003).

The transmembrane serotonin transporter (5-HTT) is a key regulator of 5-HT concentrations and governs the intrasynaptic reuptake of 5-HT into the presynaptic neuron, where it can be degraded or stored for subsequent release (Homberg & Lesch, 2011). The 5-HTT determines the magnitude and duration of extracellular 5-HT levels and is the initial target for SRI antidepressant medication (Lesch & Gutknecht, 2005). Serotonin reuptake inhibitors consist of two classes of antidepressants: selective serotonin reuptake inhibitors (SSRIs) (including citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, and sertraline), and selective norepinephrine

reuptake inhibitors (SNRIs) (including desvenlafaxine, duloxetine, milnacipran, and venlafaxine). Both classes are used to manage antenatal mood disturbances (Cooper et al., 2007) and will be referred to as SRIs. The mechanism of action is similar in both SSRIs and SNRIs, and to date there is no evidence that SRI type has a variable impact on fetal behavior (Mulder, Ververs, de Heus, & Visser, 2011; Rurak et al., 2011).

Maternal SRI treatment during pregnancy is thought to alter central fetal 5-HT levels as SRIs readily cross the placenta and blood–brain barrier (BBB) (Kim et al., 2006; Rampono et al., 2009). However, there is a paucity of comparative data with respect to transplacental SRI drug passage. Given that SRI use in pregnancy involves chronic use, SRIs are likely present at steady-state in terms of drug disposition. Thus, the relationships between fetal and maternal drug concentrations are largely determined by the ability of the fetus to metabolize the drug. For example, evidence for the stereoselective disposition and reduced ability to metabolize fluoxetine compared to paroxetine and other SRIs was shown in both pregnancy and the postpartum period in the mother, infant, and breast milk (Kim et al., 2006). Furthermore, evidence examining 15 human placentas immediately following delivery has suggested that citalopram may result in less fetal exposure than fluoxetine (Heikkinen, Ekblad, & Laine, 2002). A more recent study evaluating drug and metabolite concentrations for citalopram, escitalopram, sertraline, fluoxetine, fluvoxamine, paroxetine (SSRIs), and venlafaxine (SNRI) in the mother and cord at birth reported a linear transfer of both parent drug and active metabolites from the maternal to fetal circulation (Rampono et al., 2009). While this study was limited by a very small sample size, (Rampono et al., 2009) found that sertraline and its metabolite (*N*-desmethylsertraline) had lower cord–maternal ratios (0.33 and 0.4, respectively) than the other SSRIs (cord–maternal ratios of 0.7–0.86).

In terms of crossing the BBB, there are no human studies reporting trans BBB transfer during fetal periods. Using an analogous animal model based on similarities in the ontogenesis of the BBB, an examination of diphenhydramine (a potent histamine H_1 receptor antagonist) concentrations in the cerebral spinal fluid (CSF), extracellular fluid (ECF) and plasma in fetal, newborn and adult sheep found similar concentrations between the CSF and ECF compartments (Au-Yeung, Riggs, Gruber, & Rurak, 2007). In addition, Au-Yeung et al. (2007) reported that the brain–plasma drug ratios were significantly higher in fetal and postnatal lambs compared to adult sheep.

In human studies, brain biomarkers found in blood have been used as proxy measures of what might reflect blood-brain transfer. Neurobehavioral neonatal disturbances have been associated with measures reflecting central serotonergic levels in utero, specifically, levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) (Laine, Heikkinen, Ekblad, & Kero, 2003). Laine et al. (2003) reported that SRI-exposed infants had significantly lower cord blood 5-HIAA concentrations compared with a control group. A significant inverse correlation was also seen between the serotonergic symptom score—postnatal adaptation—and umbilical vein 5-HIAA concentrations in the SRI-exposed infants. SRI exposed neonates have lower cord blood levels of a biomarker of early brain maturation and central serotonergic function (i.e., astroglial-specific calcium binding protein, S100B) (Pawluski, Galea, Brain, Papsdorf, & Oberlander, 2009). Furthermore, SRI exposure has been correlated with increased norepinephrine metabolite (dihydroxyphenylglycol), increased thyroid stimulating hormone and reduced IGF-I cord blood levels (Davidson et al., 2009; Hilli et al., 2009), findings that might underlie impaired intrauterine growth in exposed neonates.

To date there is very limited evidence regarding how differences in breast milk drug levels vary with the type of SRI. Although breast milk as a route of postnatal exposure is much less significant than placental transfer in the fetus (Oberlander et al., 2005), a detailed review and pooled analysis including lactating mothers, breast milk and nursing infants revealed that SRI levels are present in breast milk (Weissman et al., 2004). These data revealed that breastfed infants

exposed to paroxetine and sertraline were unlikely to develop detectable serum drug levels, while infants exposed to fluoxetine through breast milk were more likely to develop elevated levels of the drug. The data on citalopram were limited but suggested that some infants developed quantifiable serum levels of the drug. More recent research has suggested that sertraline and paroxetine have the best safety profiles for use during breastfeeding (Davanzo, Copertino, De Cunto, Minen, & Amaddeo, 2011; Lanza di Scalea & Wisner, 2009).

In addition to maternal SRI dosage and serum concentrations, fetal drug exposure is determined by other factors such as fetal gene variants associated with placental transporter proteins that may influence the rate of placental drug transfer (Bonnin et al., 2011). Importantly, maternal treatment with SRI antidepressants occurs in the context of antenatal mood disturbances, which also affect levels of 5-HT during critical periods of neurodevelopment. Thus, distinguishing the impact of SRIs from the underlying maternal mood disturbances presents an immense challenge to all observational studies of fetal effects of SRI exposure (Bosco et al., 2010). Whether SRI antidepressant exposure or the underlying maternal mood disorder affect central fetal 5-HT concentrations, given 5-HTs diverse roles in fetal development, it is plausible that early alterations in serotonergic signaling could influence fetal brain development and thus affect neurobehavioral outcomes (Homberg, Schubert, & Gaspar, 2010).

Animal Models of Altered Early 5-HT Transmission

The effects of early changes in 5-HT signaling have been studied in animal models using pharmacological blockade of 5-HTT using SRI antidepressants (Maciag et al., 2006; Maciag, Williams, Coppinger, & Paul, 2006) or where 5-HTT is genetically absent, a condition resembling complete blockade of 5-HT reuptake (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004). For example, high central 5-HT levels during early postnatal rodent development (akin to a human third trimester) caused permanent axonal connection deficits in the somatosensory cortex and lateral geniculate nucleus (Gaspar et al., 2003; Homberg et al., 2010), and altered neuronal dendritic branching, elongation and pruning (Liao & Lee, 2011; Zheng et al., 2011). Importantly, studies with animal models suggest that a 5-HTT blockade, whether genetically or pharmacologically driven, leads to lasting behavioral, neurophysiological and neuroanatomical alterations (Homberg et al., 2010; Oberlander, Gingrich, & Ansorge, 2009; Olivier, Blom, Arentsen, & Homberg, 2011; Simpson et al., 2011).

Preclinical studies suggest that in utero SRI exposure alters fetal behaviors and physiology well before the gestational exposure ends. Using a sheep model, in utero SRI exposure decreased uterine blood flow and oxygen saturation, and altered the fetus' behavioral state reflected in increased quiet sleep and decreased rapid eye movement (REM) sleep and decreased fetal breathing movements (Morrison et al., 2004; Morrison, Chien, Gruber, Rurak, & Riggs, 2001; Morrison, Chien, Riggs, Gruber, & Rurak, 2002). Increased prenatal fetal plasma adrenocorticotropic hormone (ACTH) and cortisol surges also have been reported (Morrison et al., 2001, 2002, 2004). In newborn lambs exposed to fluoxetine for 12 days in late gestation, increased newborn activity during the first 2 weeks after birth was observed (Nguyen, 2013). However, findings from this sheep model showed no changes in cardiovascular, metabolic, endocrine, or behavioral functions in the newborn lambs (~4 days old) with acute fluoxetine IV injection. In addition, lambs exposed to fluoxetine in utero showed low or undetectable plasma fluoxetine and norfluoxetine concentrations. These findings suggest that acute toxicity or withdrawal effects may not be the mechanisms underlying poor neonatal adaptation in SRI exposed human infants (Nguyen, 2013).

The effects of SRI exposure on brain development have also been studied using a rodent model. Offspring of dams administered fluoxetine for 14 days during pregnancy displayed a significant decline in both the cell count in the nucleus accumbens and serotonin transporter-like immunoreactivity in the raphe nucleus, suggesting that key brain regions responsible for the reward

response systems and serotonergic pathway are impacted by in utero SRI exposure (Forcelli & Heinrichs, 2008). Using rodent models akin to the human third trimester, early postnatal SRI exposure reduced novelty exploration and sexual activity, and increased immobility, sleep abnormalities and anhedonia (Ansorge et al., 2004; Lee, 2009; Maciag, Williams et al., 2006; Popa, Léna, Alexandre, & Adrien, 2008). These effects were associated with alterations in brain structure and function including reduced dendritic span and complexity in the somatosensory cortex (Lee, 2009), decreased synthesis of tryptophan hydroxylase (a key rate limiting enzyme for 5-HT synthesis) in the dorsal raphe, and decreased 5-HTT expression in the cortex (Maciag et al., 2006). However, not all outcomes associated with early SRI exposure using rodent models reflect developmental disturbances. Increased locomotor activity and spatial task ability have been observed in adult rats exposed to citalopram from postnatal days 8–21 (Maciag et al., 2006).

The neuroanatomical and functional consequences of changing 5-HT levels depend on the timing (critical periods) and direction (increased or decreased) of the developmental exposure, which may differ from the impact of an acute exposure in a mature organism (Ansorge, Hen, & Gingrich, 2007; Kalueff et al., 2010). In a rodent model, SRI exposure over a specific early postnatal period (postnatal days 4–21) of development is also associated, paradoxically, with reduced exploratory behavior, and depressive and anxiety-related behaviors in adulthood. These effects mimic the very effects of genetic 5-HTT inactivation (i.e., gene knockout models leading to the absence of the transporter), suggesting that increased serotonergic signaling during a developmentally critical period predisposes to subsequent affective disturbances (Ansorge et al., 2004; Ansorge, Morelli, & Gingrich, 2008; Gobbi, Murphy, Lesch, & Blier, 2001; Lira et al., 2003). Interestingly, in the long term, SRI-exposed animals demonstrate a decrease in 5-HT levels—possibly via activation of inhibitory receptors (i.e., 5-HT_{1a}) (Hensler, 2006). Such alterations in 5-HT signaling are evident at the neurostructural and behavioral levels, and in abnormal circuitry and cortical network functions (Simpson et al., 2011).

Fetal Effects of SRI Exposure: Human Findings

Gross structural neuroteratogenic effects following in utero SRI exposure have not been identified in humans; however, evidence pointing to functional behavioral disturbances is emerging (Oberlander, 2012). Fetal SRI exposure varies greatly (Kim et al., 2006; Rampono et al., 2009) and is a reflection of key maternal, placental, fetal metabolic, genetic, and pharmacological factors (Shea, Oberlander, & Rurak, 2012). During gestation, well before SRI exposure ends, changes in fetal neurobehavioral development have been observed including disrupted non-REM sleep and increased motor activity early in gestation followed by poor inhibitory motor control during non-REM quiet sleep near term (Mulder et al., 2011). Reduced fetal brain flow indices and heart rate variability also have been observed in the third trimester both before and after a typical daily maternal SRI dose (Rurak et al., 2011), even when controlling for maternal mood disturbances. Using fetal actocardiography with ultrasound observations, increased motor movements and reduced fetal breathing have been reported in SRI exposed fetuses (Salisbury, Ponder, Padbury, & Lester, 2009). In late gestation, SRI exposed fetuses were found to have reduced middle cerebral artery cross-sectional area before and following a typical daily maternal SRI dose (Rurak et al., 2011), suggesting an early and sustained medication-related effect. Moreover, altered cord red blood cell indices (increased hemoglobin concentration and increased hematocrit) at birth suggest that SRI exposure might be associated with altered fetal hypoxia and hypoxia-induced altered blood flow (Rurak et al., 2011). Whether these changes persist and represent the early origins of altered neonatal or childhood neurobehavior remains to be studied. Together these findings support the notion that the effects of SRIs on fetal behavior and function are a reflection of early alterations in neurobehavior, in contrast to the view that poor behavioral disturbances are associated with the acute cessation of SRI drug exposure at birth, pharmacological toxicity (Oberlander et al., 2004), or excess of 5-HT (Laine et al., 2003).

Impact of Prenatal Maternal Mood Disturbances

The impact of fetal SRI exposure cannot be effectively determined without considering the effects of the underlying indication for the drug treatment, and the maternal mood disturbance, which has been shown to impact fetal physiology and behavior. Many studies examining outcomes of in utero SRI exposure frequently yield highly conflicting findings showing both increased or no risk to neonatal/infant outcomes (Homberg & Lesch, 2011). This confusion can be explained at least partially as the result of the key challenge of "confounding by indication" (Bosco et al., 2010). Failure to effectively treat maternal depression and/or anxiety can lead to compromised prenatal care, increased risk of obstetrical complications, self-medication and/or substance abuse, as well as exposure to the illness itself. Maternal mental health that leads to SRI treatment also affects neurodevelopment (Glover, 2011) and fetal serotonergic signaling (Field, 2004; Field et al., 2008). Importantly, SRI treatment does not effectively treat maternal mental health disturbances for all women, and many women being treated with SRI antidepressants continue to experience depression and anxiety. Regardless of whether women continue or discontinue their antidepressant during pregnancy, Yonkers et al. (2011) showed that both groups had a similar risk of a major depressive episode in pregnancy (approximately 16 %).

There is evidence that antenatal maternal stress disrupts fetal neurobehavioral development (DiPietro, Hodgson, & Costigan, 1996; Tronick & Reck, 2009) and alters behavioral reactivity in utero (Allister, Lester, Carr, & Liu, 2001; Monk et al., 2000).

Perinatal maternal mood disturbances have been associated with reduced birth weight, and increased risks for prematurity (Glover, 2011; Talge, Neal, & Glover, 2007). Furthermore, antenatal exposure to maternal depressed mood appears to be reflected in newborns and has been associated with neonatal irritability, atypical frontal EEG patterns, reduced vagal tone, elevated cortisol and norepinephrine, and lower dopamine and 5-HT levels (Talge et al., 2007). Beyond the newborn period, antenatal maternal anxiety predicts infant temperament and attention regulation during the first year of life (Austin, Hadzi-Pavlovic, Leader, Saint, & Parker, 2005; Davis et al., 2007; Pluess et al., 2011; Talge et al., 2007), even when accounting for postnatal maternal psychological state. After controlling for obstetric risk, psychosocial disadvantage, and postnatal maternal mood, antenatal maternal anxiety continues to influence cognitive, behavioral, and emotional outcomes well into childhood (Talge et al., 2007). While the exact mechanisms by which antenatal anxiety/stress influence fetal brain development remain unclear, there is sufficient animal or human evidence to suggest that early life adversity predisposes to poor mental health and stress adaptation across the life span (Charney, 2004; Charney & Manji, 2004).

Even small changes in maternal disposition among euthymic mothers may be associated with differences in blood flow, fetal heart rate variability, and diurnal fetal patterns. We examined whether mothers positive and negative affect were associated with fetal vascular and heart rate changes at 36 weeks of gestation in euthymic mothers (Hanley, Rurak, Lim, Brain, & Oberlander, 2014). Negative affect reflects an individual's tendencies to express feelings like anger, contempt, shame, fear, and depression in response to life's stressors (Watson & Pennebaker, 1989). On the other hand, individuals' with high positive affect (which reflects an individual's enthusiasm, activity, control, and commitment) seem able to maintain a positive outlook both over time and in various situations (Bood, Archer, & Norlander, 2004). We found that mothers who reported high levels of negative affect showed reduced uterine artery flow, decreased fetal heart rate variability, an altered diurnal pattern, and decreased uterine artery cross-sectional area compared to mothers who reported low levels of negative affect. In contrast, mothers with low positive affect had a steeper diurnal pattern in fetal heart rate accelerations and decreased uterine artery mean velocity flow than mothers with high positive affect. While this was a small cohort study and these results need further investigation,

it suggests the possibility that even in the absence of an Axis I Major Depressive Disorder (MDD), maternal affect may have an impact on fetal and uterine physiology.

The Impact of Fetal SRI Exposure: Postnatal Findings in Humans

Neonatal Neurobehavioral disturbances: A postnatal adaptation syndrome (PNAS) or "withdrawal-like condition" has been widely reported and could be regarded as an extension of in utero behavioral changes (Moses-Kolko et al., 2005). PNAS typically includes some combination of the following symptoms: respiratory distress (Chambers, Johnson, Dick, Felix, & Jones, 1996; Davis, Rubanowice, et al., 2007; Diav-Citrin et al., 2008; Hemels, Einarson, Koren, Lanctôt, & Einarson, 2005), feeding difficulty (Ansorge et al., 2004; Oberlander, 2012), jitteriness (Oberlander, 2012), temperature instability (Oberlander, 2012; Tronick & Reck, 2009), sleep problems (Hensler, 2006), tremors (Laine et al., 2003), shivering (Laine et al., 2003), restlessness (Laine et al., 2003), convulsions (Davis et al., 2007; Kallen et al., 2004), jaundice (Costei, Kozer, Ho, Ito, & Koren, 2002; Oberlander et al., 2006), rigidity (Laine et al., 2003), and hypoglycemia (Chambers et al., 1996; Costei et al., 2002; Davis et al., 2007; Kallen et al., 2004). PNAS occurs in about 30 % of SRI exposed newborns (Levinson-Castiel, Merlob, Linder, Sirota, & Klinger, 2006; Oberlander et al., 2004) with a particular risk associated with SRI exposure during the last trimester of pregnancy (Moses-Kolko et al., 2005).

Studies on the impact of timing of exposure are few and logistically challenging (i.e., sample size, infrequent outcomes, and controlling for confounders that influence timing). Women treated with SSRIs late in pregnancy had a higher frequency of delivering SGA (small for gestational age) infants, and women receiving non-SSRI antidepressants were more likely to deliver premature and SGA offspring (Toh et al., 2009). Further, neonates with third trimester SRI exposure (often referred to as "late exposure" in the literature) have been reported to be at an increased risk for a special care nursery admission (Chambers et al., 1996; Cohen et al., 2000) and respiratory difficulty (Costei et al., 2002; Kallen et al., 2004; Oberlander et al., 2004) compared to neonates with first or second trimester or no SRI exposure. Importantly, for the most part, PNAS symptoms are mild and self-limited. The average time of onset for PNAS symptoms ranges between birth and 3 days of age and lasts for up to 2 weeks (Austin, 2006).

PNAS symptoms resemble a neonatal SRI drug withdrawal associated with an acute cessation of drug exposure, a pharmacological condition well recognized in adults, yet the underling etiology and clinical significance remains unclear (Warner, Bobo, Warner, Reid, & Rachal, 2006). Moreover, these behaviors also could be a continuation of fetal behavioral disturbances secondary to an acute fetal drug exposure or a reflection of sustained altered brain development that spans gestation. The severity of increased motor activity and tremors (Moses-Kolko et al., 2005), and altered stress regulation (Oberlander et al., 2002) has been associated with increased SRI drug levels (Oberlander et al., 2004) and pharmacogenetic metabolic factors (Laine et al., 2003), suggesting a potential pharmacologic toxicity. Further supporting this hypothesis, fluoxetine has the longest half-life of the commonly used SRIs and the lowest risk of withdrawal among adult patients (Coupland, Bell, & Potokar, 1996), yet maternal treatment in late pregnancy is associated with PNAS (Moses-Kolko et al., 2005). A dose dependent relationship with the severity of PNAS symptoms has been observed (Levinson-Castiel et al., 2006).

PNAS symptoms also are thought to reflect neurobehavioral changes associated with measures reflecting sustained central serotonergic levels in utero, specifically, levels of 5-HIAA (5-HT metabolite) (Laine et al., 2003). In this sense, PNAS symptoms could reflect fetal disturbances that predate birth. How these findings play out in terms of infant and child health risk over the first few years of life remains unclear; however, emerging evidence points to links between PNAS and development in early childhood. In SRI

exposed children, externalizing behaviors were associated with increased cord drug levels, particularly in children with a history of neonatal withdrawal symptoms (Oberlander et al., 2007). Further, in a recent study, Klinger et al. (2011) reported increased social behavioral disturbances in 2–6 year olds with a history of PNAS. Determining why some, but not all neonates are at risk for these neurobehavioral disturbances is a key question.

Risk for congenital malformations and cardiac defects using a case control study design (Louik, Lin, Werler, Hernández-Díaz, & Mitchell, 2007) were increased with first trimester paroxetine exposure (Jimenez-Solem et al., 2012; Knudsen, Hansen, Garne, & Andersen, 2014), while in other studies no association with increased first trimester exposure have been reported (Oberlander et al., 2008; Wichman et al., 2009). Importantly, confounding by indications associated with the use of SSRIs during pregnancy, such as socioeconomic status and maternal mood disturbances, may have an impact on risk (Jimenez-Solem et al., 2012; Oberlander et al., 2006). Further, rather than a particular point in time, length of prenatal SSRI use appears to affect neonatal and infant behavior (Casper et al., 2011; Oberlander, Warburton, Misri, Aghajanian, & Hertzman, 2008).

Genetic variations have been considered to play a role in moderating the impact of in utero SRI exposure, similar to differences in clinical effects of SRIs in adults (Pollock et al., 2000). Allelic variations for 5-HTT may influence the risk for PNAS, suggesting a gene-environment interaction. A 44 base pair insertion/deletion in the 5-HTT gene-linked polymorphic region (*5-HTTLPR*) produces a long (l) or short (s) allelic variant, with the long variant transcriptionally more efficient, resulting in higher 5-HTT expression and function (Homberg & Lesch, 2011). In SRI exposed neonates, two short alleles (ss) for the transporter were found to be associated with reduced 5-min Apgar scores, increased jitteriness, and increased muscular tone (Oberlander et al., 2008). In addition, compared to non-exposed infants, birth weight was lower in SRI exposed infants with an ls allele, and risk for respiratory symptoms (respiratory distress and tachypnea) was higher in SRI exposed infants with an ll allele (Oberlander et al., 2008).

Stress Regulation

In utero SRI exposure has also been shown to alter early stress regulation. Preclinical and human findings point to an in utero SRI-related "programming" effect on both sympathetic adrenal medullary (SAM) and hypothalamic pituitary adrenal (HPA) stress systems. However, some of these effects only become apparent in a particular postnatal maternal care-giving context. In response to an acute painful event, the duration of facial action and cardiac responses—particularly parasympathetic cardiac activity—are shorter and less intense in exposed neonates (Oberlander et al., 2002). Altered pain reactivity persists at 2 months of age, after controlling for drug levels and maternal mood (Oberlander et al., 2005).

In early infancy in utero SRI exposure affects cortisol levels and its binding protein, corticosteroid-binding globulin (CBG) (Pawluski, Brain, Underhill, Hammond, & Oberlander, 2011). Exposed neonates had increased CBG levels, particularly after vaginal delivery, though cord cortisol levels did not vary with in utero SRI exposure or antenatal maternal mood. These findings are not surprising given that serotonergic neurons projecting from the raphe nuclei to brain regions that affect motor development also affect sleep-awake function (Fuller, Gooley, & Saper, 2006) and regulate autonomic control (Bairy, Madhyastha, Ashok, Bairy, & Malini, 2007). Thus, changes in fetal 5-HT signaling may alter developmental processes that influence sleep, motor, and stress regulation.

Interestingly, the effects of prenatal SRI exposure on stress regulation may be apparent only in the presence of specific postnatal challenges long after delivery. At 3 months, SRI exposed infants had a reduced diurnal change in salivary cortisol, possibly suggesting that prenatal SRI exposure affects the developing HPA system via altered serum neonatal CBG levels (Pawluski et al., 2011). This also may be reflected in altered HPA

stress patterns and lower early evening basal cortisol levels in SRI exposed infants (Oberlander et al., 2008). In response to a non-noxious challenge at 3 months of age, SRI exposed and non-exposed infants exhibited similar salivary cortisol levels. However, when infant feeding status was considered, differences associated with SRI exposure emerged. Specifically, compared with breastfed SRI and breastfed non-SRI exposed infants, non-SRI exposed/non-breastfed infants showed a blunted post-stress cortisol pattern (Oberlander et al., 2008), possibly reflecting a self regulatory capacity that might heighten a senstivity to maternal caregiving.

Child Development and In Utero SRI Exposure

Motor Development

Recently research has begun to report differences in child development following in utero SRI exposure that might reflect long-term consequences of altered fetal neurodevelopment. These results appear to be consistent with animal studies reporting delayed motor development in SRI exposed mice (Bairy et al., 2007). The findings are not all together unexpected given the common neurodevelopmental role of 5-HT (Kalueff et al., 2010). Research using animal models has suggested that the links between in utero SRI exposure and motor control might reflect an early effect of muscle tone development under serotonergic control (Jacobs & Fornal, 1999). Poorer motor performance has been associated with impaired neurodevelopment in fluoxetine treated rats (Zheng et al., 2011), and alterations in muscle tone control have been reported as effects of SRIs in human infants (Laine et al., 2003). Human studies on motor development have presented conflicted findings. Typical mental and psychomotor development has been reported in some (Misri et al., 2006; Nulman et al., 1997, 2002), but not all studies (Casper et al., 2003; Hanley, Brain, & Oberlander, 2013; Mortensen et al., 2003; Pedersen, Henriksen, & Olsen, 2010). Two studies examining development using the Bayley Scales of Infant Development (BSID) have shown no difference in total BSID score (Nulman et al., 1997, 2002), while four others have reported lower scores on the motor index of the BSID in exposed children (Casper et al., 2003, 2011; Galbally, Lewis, & Buist, 2011; Hanley et al., 2013). Further research is needed in this area. We recommend that specific measures designed to assess motor development need to be collected in a longitudinal cohort of children in order to add to our understanding of the impact of prenatal SRI exposure on long-term motor development. Importantly, while differences in motor development were statistically significant, the clinical and long-term developmental consequences remain to be determined.

Risks for Autism Spectrum Disorder

Beyond motor development, increasing attention is being paid to the association between 5-HT during fetal brain development and autism spectrum disorder (ASD) in the child. Altering 5-HT levels during critical periods of development may affect brain areas in which serotonergic innervation is involved in regulating communication and social behavior—two key components that characterize ASD. As such, these alterations could underlie psychiatric and/or developmental conditions such as ASD (Jørgensen, Kjaer, Knigge, Møller, & Warberg, 2003).

The serotonin hypothesis of autism proposes that autism may in part have its origins in early dysfunctional 5-HT signaling. The developmental hyperserotonemia (DHS) model was proposed following a report that higher levels of 5-HT were detected in a third of patients with ASD (Whitaker-Azmitia, 2005). The DHS model hypothesizes that before the BBB is fully formed, high levels of 5-HT in maternal blood could enter the fetal developing brain and lead to a loss of serotonergic nerve terminals through negative feedback, blunting long term 5-HT signaling. Hyperserotonemia is the most consistent neurochemical change associated with ASD (Anderson, Horne, Chatterjee, & Cohen, 1990; Cook et al., 1993); it has been observed in first-degree relatives (Cross

et al., 2008) and is associated with risk of ASD within families (Abramson et al., 1989; Piven et al., 1991). Considering the DHS model and the fact that SRI antidepressants increase how much and how long extracellular 5-HT remains active and available, altering fetal central 5-HT levels (Weikum, Oberlander, Hensch, & Werker, 2012), concerns have been raised about how such early altered 5-HT signaling contributes to ASD risk.

Using animal models, manipulating prenatal 5-HT levels induce numerous neurological and behavioral abnormalities similar to those observed in ASD patients (Green et al., 2001; Modahl et al., 1998; Nelson et al., 2001). To model DHS in animals, rodents exposed to a 5-HT agonist during a gestational period analogous to the third trimester in humans, were observed to have changes in serotonergic receptors and columnar development in the cortex (Casanova, Buxhoeveden, Switala, & Roy, 2002; Whitaker-Azmitia, 2005). Changes in the amygdala and hypothalamus, brain regions that regulate mood, stress, emotion, and social responsiveness (all of which are dysregulated in ASD patients) showed higher levels of calcitonin gene-related peptide (Nelson et al., 2001) and lower levels of oxytocin (a peptide involved in bonding and social behavior), two key findings observed in humans with ASD (Green et al., 2001; Modahl et al., 1998). At a behavioral level, rodents exhibited behaviors similar to the clinical presentation of autism such as decreased social bonding, social interactions (McNamara, Borella, Bialowas, & Whitaker-Azmitia, 2008), sensory hyper-responsiveness, seizures, and motor impairment (Whitaker-Azmitia, 2005).

Direct evidence that links altered serotonergic signaling with an increased ASD risk in humans remains limited. Two recent epidemiological studies offer new lines of evidence suggesting that in utero SRI exposure may be one of the contributing factors. Researchers at Kaiser Permanente (California) used nested case-control methods to determine if exposure to SRIs in utero was more common among children aged 0–2 years with a diagnosis of ASDs than in typically developing comparison children (Croen et al., 2011; Rai et al., 2012). The researchers reported a twofold increase in exposure to SRIs among case children (6.7 % vs. 3.3 % in controls), even when controlling for maternal psychiatric history, demographics and co-morbidities. However, it is not clear if diagnostic codes for autism available in administrative datasets are valid indicators of autism, and the authors did not refer to any validation studies done on these data, suggesting possible diagnostic uncertainty. A second epidemiological study from Sweden used valid diagnoses of ASD. Rai et al. (2012) reported that maternal depression during pregnancy, and/or exposure to either SRIs or a non-selective monoamine reuptake inhibitor antidepressant (i.e., tricyclic antidepressants) was associated with an increased risk of an ASD. However, as with all observational research examining the effects of SRI use during pregnancy, these findings are susceptible to "confounding by indication" whereby distinguishing the impact of maternal mood disturbances and the medication used to treat it, remains a significant methodological challenge. This concern is increased by the fact that an increased risk of ASD also has been reported among children born to mothers who were depressed during pregnancy (Daniels et al., 2008; Piven & Palmer, 1999). Two recent studies have suggested that the association between SRI exposure in utero and ASD can be entirely explained as confounding by indication. Both Clements et al. (2014) and Hviid, Melbye, and Pasternak (2013) failed to identify a statistically significant increased risk of ASD among children who were exposed to SRIs in utero, but they did identify increased risk in children whose mothers used SRIs prior to pregnancy, suggesting that the risk was the underlying maternal psychopathology rather than the medicine. While these may be intriguing outcomes, the lack of follow-up beyond early childhood, the ongoing difficulty distinguishing maternal mood from medication effects, and the paucity of directly validated individual specific ASD diagnoses in these studies means that this area warrants further study.

Summary

In utero SRI exposure is common and neonatal behavioral outcomes following exposure vary greatly. Emerging findings suggest that SRI effects may be evident during gestation. In utero SRI exposure appears to influence fetal brain blood flow and neurobehavior. Moreover, fetal biobehavioral disturbances associated with such in utero exposure might predict altered developmental processes and subsequent long-term developmental risk. Preclinical findings highlight three key points regarding changes in 5-HT signaling associated with early SRI exposure that have critical implications for our understanding of human fetal development. First, both behavioral and physiological effects are apparent even before acute in utero exposure ends and these effects persist beyond birth. Second, altered 5-HT levels—either from genetic variations or pharmacologically driven following SRI exposure—induces a 5-HT reuptake blockade that appears to have a long-term impact on behavior such as anxiety and depression-like symptoms that extend into adulthood. Third, altered stress regulation has been associated with in utero SRI exposure which may have implications for mental and physical health across the life span. Together these findings suggest that in utero SRI exposure that alters early 5-HT auto-inhibitory feedback, leading to high serotonergic tone during developmentally sensitive periods, alters the maturation and function of the 5-HT system (Ansorge et al., 2004). Emerging findings in humans appear to point to similar in utero biobehavioral effects as well as long-term developmental and behavioral sequelae. Together these findings add to mounting concern about the use of SRI antidepressants during pregnancy and uncertainty for mothers and their clinicians.

While in utero SRI exposure alters central 5-HT levels, developmental outcomes do not necessarily reflect a solitary effect that can be easily attributed to one causal factor (i.e., maternal mood, genetics, or antidepressants). Rather, outcomes in this setting represent an interplay of psychological, pharmacological, genetic and social factors related to both mother and her child. Antidepressants might be prescribed during pregnancy with the expectation of optimizing infant health coupled with associated improved maternal mood; however, current evidence suggests that not all women respond to treatment and infants may continue to be at risk as maternal pharmacotherapy might not "buffer" or protect them from antenatal or postnatal maternal mood disturbances. It is important to recognize that this is a context of developmental vulnerability as well as neuroplasticity. Therefore, identifying mothers and their infants who might benefit from in utero maternal SRI treatment remains a key and pressing question. Longitudinal study designs that integrate both maternal and infant/child developmental perspectives should help us move away from characterizing in utero SRI exposure, maternal mood, or even genetic variations as "bad" or "harmful" and rather look at these as adversity or risk related factors that heighten or lessen vulnerability associated with early development.

From a maternal–child health perspective our task is to recognize risks arising from *both* the maternal disease and its treatment, and find ways to promote optimal child development and behavior in the context of family well-being. The decision to initiate SRI treatment during pregnancy rests with the mother and her physician carefully weighing the risks and benefits (Oberlander & Wisner, 2012). In providing antenatal treatment that requires SRI antidepressants, one needs to recognize risk characteristics that are inherent to an individual mother (and her child), in contrast to seeing them as just part of a population of prenatally treated mothers and their exposed children. There is a need to effectively diagnose and address antenatal maternal mental health with pharmacological and non-pharmacological options (cognitive/behavioral, social support, diet, housing, etc.), remembering that medications may just be one of many options available. This should include addressing the well-being of the entire family and its social context, ensuring access to affordable and appropriate health care, and laying community support. Ultimately, it might not be possible to distinguish the effects of disease from antidepressant treatment, nor may it even be necessary. What is critical is that we

recognize that multiple and ongoing "environmental pathogens" in this setting require ongoing watchful surveillance and timely interventions.

Future research needs to prioritize distinguishing the impact of maternal mood from the SRI exposure as well as working to identify the factors that contribute to both positive (e.g., maternal remission of depression, neonatal health) and negative outcomes (e.g., continued maternal depression and anxiety, PNAS syndrome) in maternal–infant pairs exposed to SRIs during the perinatal period. Improving our understanding of these critical topics will increase our ability to safely and effectively address maternal mental health disturbances during pregnancy.

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Part V

New Imaging Technologies

HDlive in the Assessment of Twin Pregnancy

19

Toshiyuki Hata, Kenji Kanenishi, Uiko Hanaoka, Rina Uematsu, and Genzo Marumo

Abstract

HDlive is a new surface-rendering mode incorporated into an ultrasound machine, which uses an adjustable light source that facilitates the ability to create lighting and shadowing effects, thereby increasing depth perception and improving image clarity. HDlive can provide clearer twin fetal images than conventional three-dimensional ultrasound. Moreover, we can clearly and easily recognize exact and precise inter-twin contacts using HDlive compared to conventional four-dimensional ultrasound. HDlive is an accurate and reliable modality for the assessment of fetal inter-twin contacts or intra-pair stimulations in twin pregnancies. In this chapter, we discuss the latest topics regarding twin fetal development, inter-twin contact and intra-pair stimulation, and twin activity level and later temperament using HDlive. HDlive may become an important technique in future behavioral research on twins and the antenatal evaluation of abnormal twin pregnancies.

Keywords

4D ultrasound • HDlive • Twin pregnancy • Fetal development • Inter-twin contact • Intra-pair stimulation • Twin activity level

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Introduction

HDlive is a new surface rendering mode incorporated into an ultrasound machine which uses an adjustable light source that facilitates the ability to create lighting and shadowing effects, thereby increasing depth perception and improving image clarity (Kagan, Pintoffl, & Hoopmann, 2011). This technique provides such extraordinarily realistic imaging of the embryo and fetus that it is almost impossible to differentiate between actual photographs and sonographic images (Merz, 2012). There have been several reports on HDlive evaluation of healthy singleton embryos and fetuses, and abnormal fetuses (Bonilla-Musoles, Raga, Castillo et al. 2013; Bonilla-Musoles, Raga, Osborne et al. 2013; Grigore & Mares, 2013; Hanaoka et al., 2014; Hata, 2013; Hata, Hanaoka et al. 2012, Hata, Hanaoka, Mashima, et al. 2013; Hata, Hanaoka, Tenkumo, 2013, Hata, Uketa, et al., 2013, Hata, Hanaoka, & Mashima, 2014, Hata, Kanenishi, et al., 2014; Kagan et al., 2011; Merz, 2012). However, there has been no report on HDlive evaluation of twin pregnancy in all three trimesters. The present chapter describes the latest state-of-the-art HDlive imaging of twin pregnancy, and makes recommendations for future research in this field.

Fetal Twin Development

In normal twin pregnancy, morphological and anatomical development of the embryo and fetus can be clearly depicted in the first and second trimesters (see Figs. 19.1, 19.2, 19.3, 19.4, 19.5, 19.6, 19.7, 19.8, 19.9, 19.10, 19.11, 19.12, 19.13, and 19.14). However, it is very difficult to depict twin fetuses in the third trimester of pregnancy because of the in utero crowding.

8–9 Weeks Gestational Age (GA)

HDlive clearly shows two embryos in two separate amniotic sacs, and two yolk sacs outside the amniotic sac in one gestational sac in monochorionic diamniotic (MD) twins at 8–9 weeks of gestation (Figs. 19.1 and 19.3). The vitelline duct is also depicted. In dichorionic diamniotic (DD) twins, each twin fetus in each gestational sac with a thick dividing membrane is evident (Fig. 19.2).

10–13 Weeks GA

HDlive allows anatomically realistic visualization of the fetus, and umbilical cord (shown in Figs. 19.4, 19.5, 19.6, 19.7, and 19.8). Fetal facial structures such as the eyes, nose, ears, and mouth are clearly evident at 12–13 weeks of gestation (Fig. 19.7). In MD twins, two fetuses in one gestational sac are evident (Figs. 19.4, 19.5, and 19.8). One large placenta and umbilical cord attachments are sometimes identified (Fig. 19.8). In DD twins, each twin fetus in a separate gestational sac with a thin dividing membrane is clearly noted (Figs. 19.6 and 19.7).

14–19 Weeks GA

All anatomical whole-body structures can be recognized early in the second trimester of pregnancy (see Figs. 19.9, 19.10, 19.11, and 19.12). The distance between the two fetuses is very small.

After 20 Weeks GA

In utero crowding of fetuses increases with advancing gestation (Fig. 19.13). Realistic faces of both twins can be noted after 20 weeks of gestation, and facial expressions are also evident (Fig. 19.14).

Inter-twin Contact and Intra-pair Stimulation

Kurjak et al. (2013) assessed the onset and frequencies of inter-twin contacts by conventional four-dimensional (4D) ultrasound in the first trimester of pregnancy. The first inter-twin

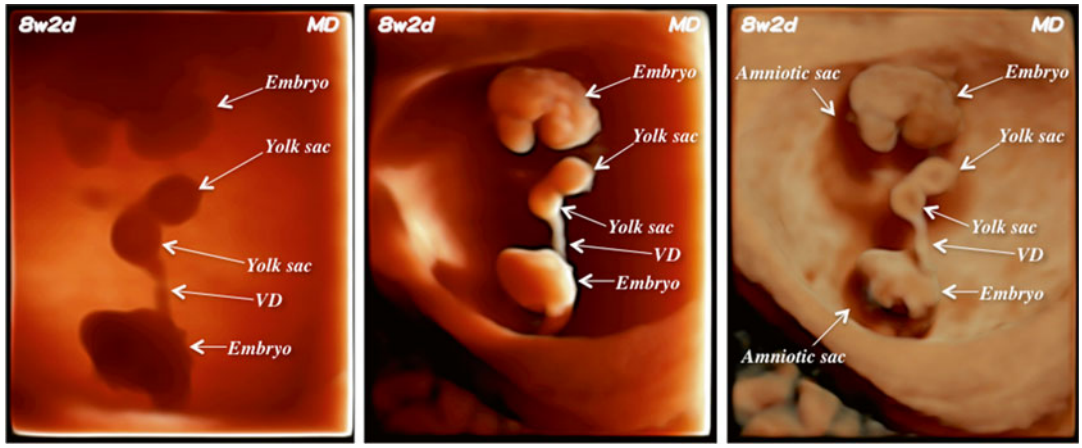

Fig. 19.1 HDlive images of a monochorionic diamniotic (MD) twin pregnancy at 8 weeks and 2 days of gestation. Two embryos in separate amniotic sacs and two yolk sacs outside the amniotic sac in one gestational sac are noted. The vitelline duct (VD) is also depicted

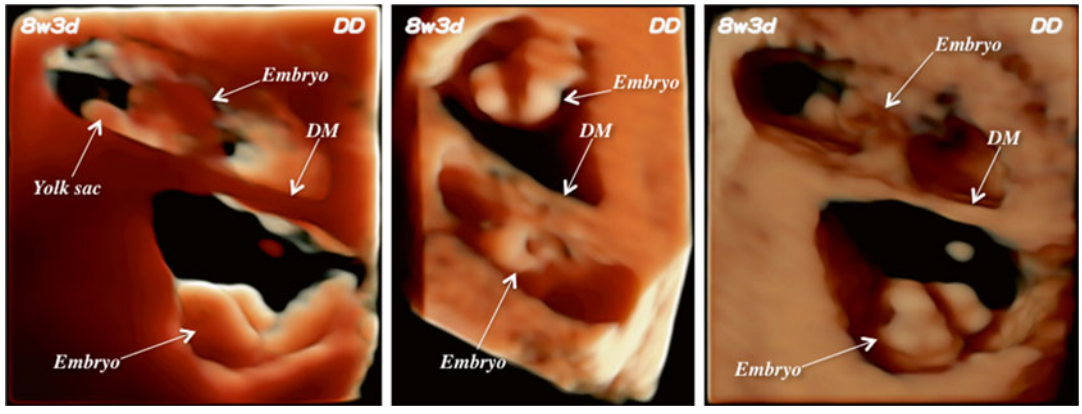

Fig. 19.2 HDlive images of a dichorionic diamniotic (DD) twin pregnancy at 8 weeks and 3 days of gestation. Each twin fetus in a separate gestational sac with a thick dividing membrane (DM) is evident

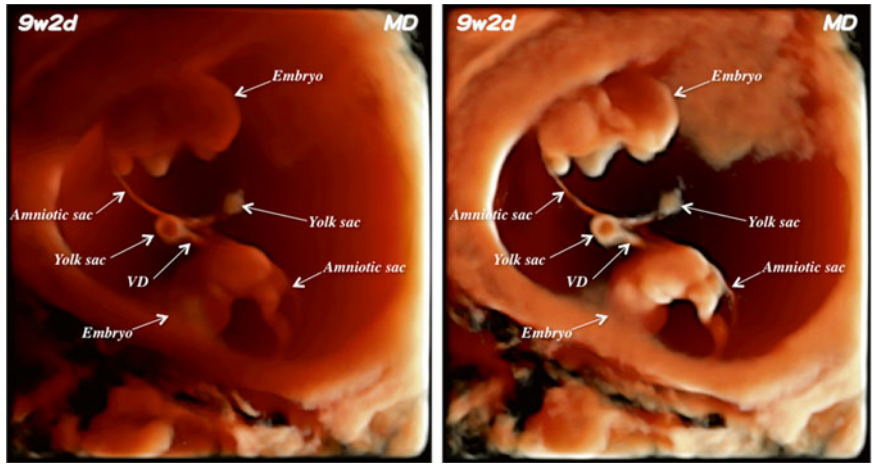

Fig. 19.3 HDlive images of a monochorionic diamniotic (MD) twin pregnancy at 9 weeks and 2 days of gestation. Two embryos in separate amniotic sacs and two yolk sacs outside the amniotic sac in one gestational sac are noted. The vitelline duct (VD) is also depicted

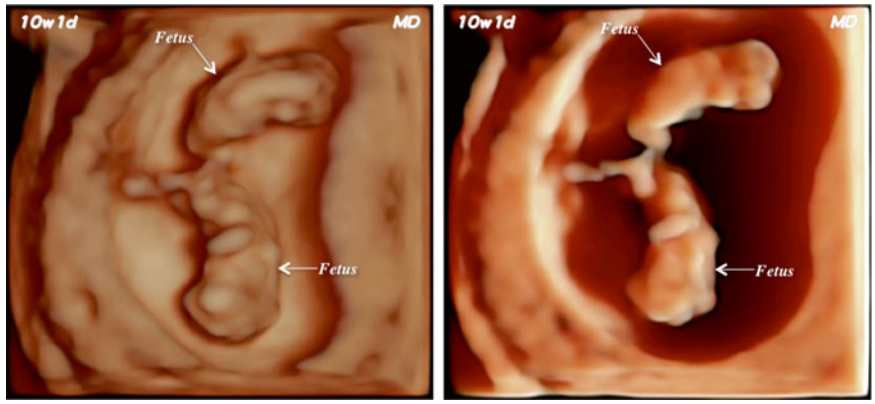

Fig. 19.4 HDlive images of a monochorionic diamniotic (MD) twin pregnancy at 10 weeks and 1 day of gestation. Two fetuses in one gestational sac are evident

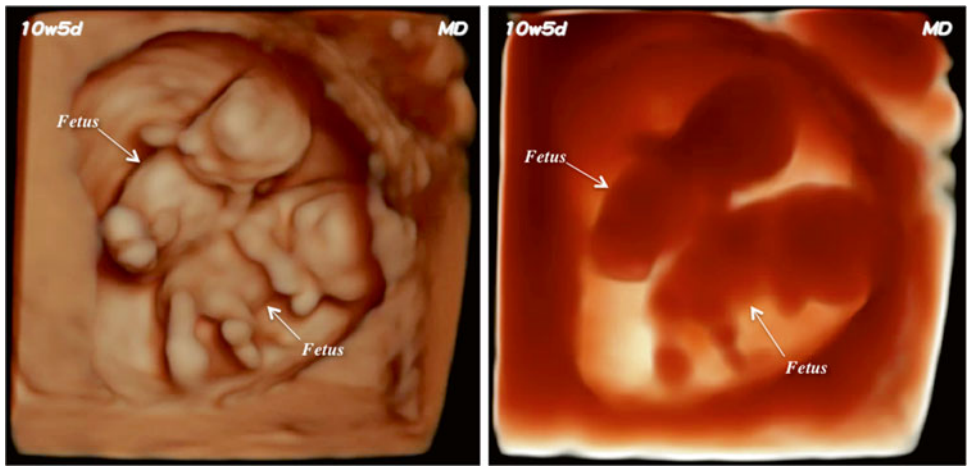

Fig. 19.5 HDlive images of a monochorionic diamniotic (MD) twin pregnancy at 10 weeks and 5 days of gestation. Two fetuses in one gestational sac are clearly shown

contacts appear at 61 postmenstrual days, while complex body movements appear at 68 postmenstrual days. The complexity of inter-twin contacts [contact lasting more than 5 s, whereby action and reaction might be repetitive (Kurjak et al., 2013)] increases from 84 postmenstrual days. With advancing gestational age, a higher frequency of movements is observed between 8 and 16 weeks of gestation. Sasaki, Yanagihara, Naitoh, and Hata (2010) evaluated the total number of inter-twin contacts between MD and DD twins using 4D ultrasound late in the first trimester of pregnancy, and a total of ten inter-twin contacts (head to head, head to arm, head to trunk, head to leg, arm to arm, arm to trunk, arm to leg, trunk to trunk, trunk to leg, and leg to leg contact)

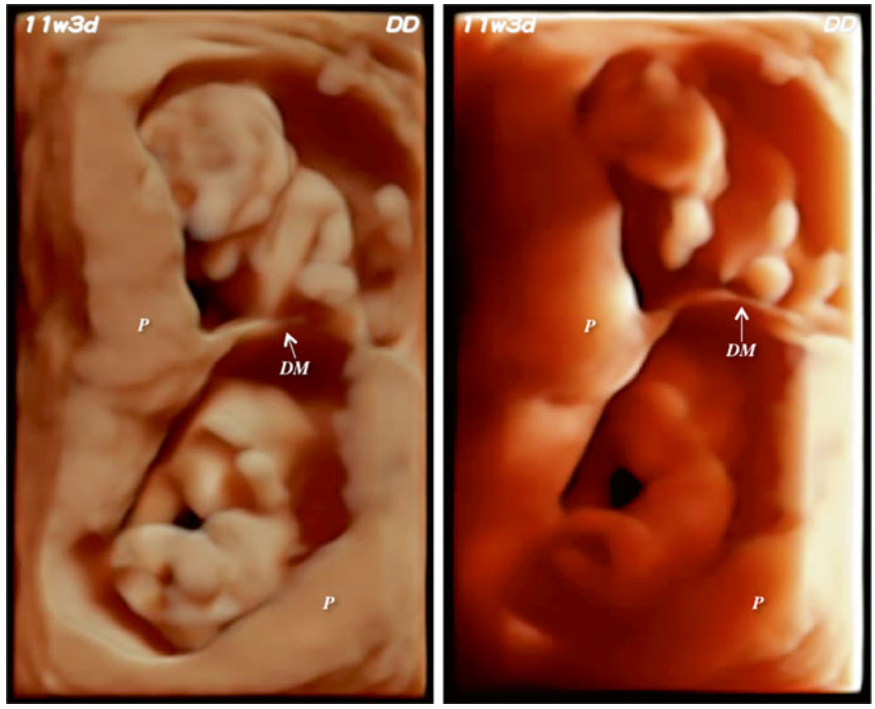

Fig. 19.6 HDlive images of a dichorionic diamniotic (DD) twin pregnancy at 11 weeks and 3 days of gestation. Each twin fetus in a separate gestational sac with a thin dividing membrane (DM) is evident. *P* placenta

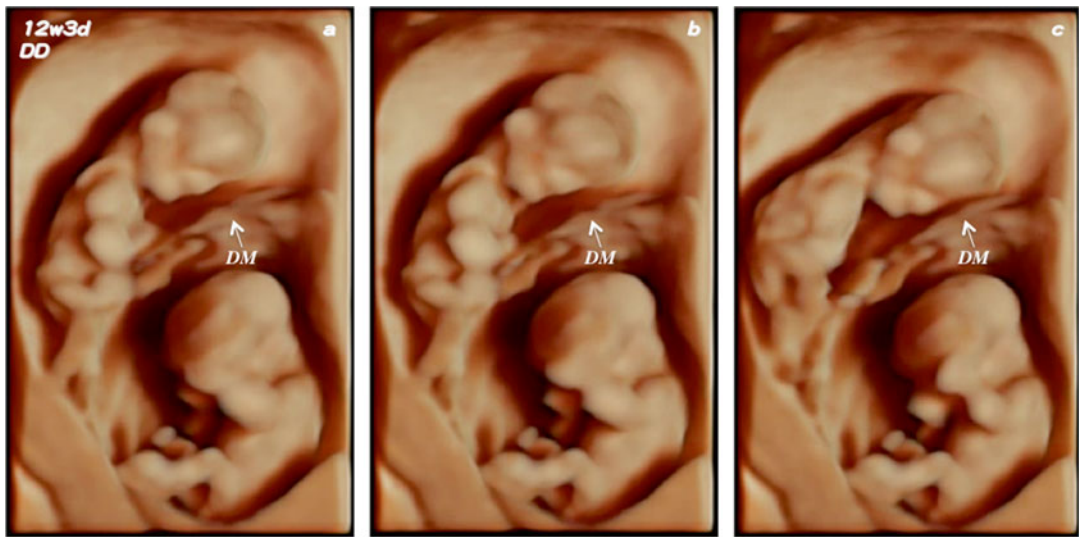

Fig. 19.7 Consecutive HDlive images of a dichorionic diamniotic (DD) twin pregnancy at 12 weeks and 3 days of gestation (**a**–**c**). Each twin fetus in a separate gestational sac with a thin dividing membrane (DM) is clearly evident. The fetal facial structures such as the eyes, nose, ears, and mouth are clearly evident at this age

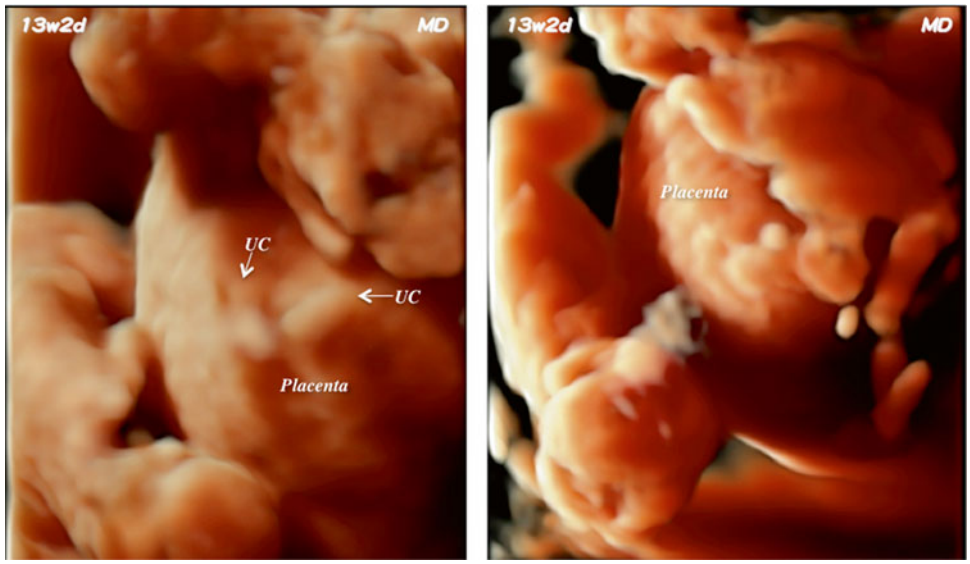

Fig. 19.8 HDlive images of a monochorionic diamniotic (MD) twin pregnancy at 13 weeks and 2 days of gestation. One large placenta and two umbilical cord (UC) attachments are clearly identified

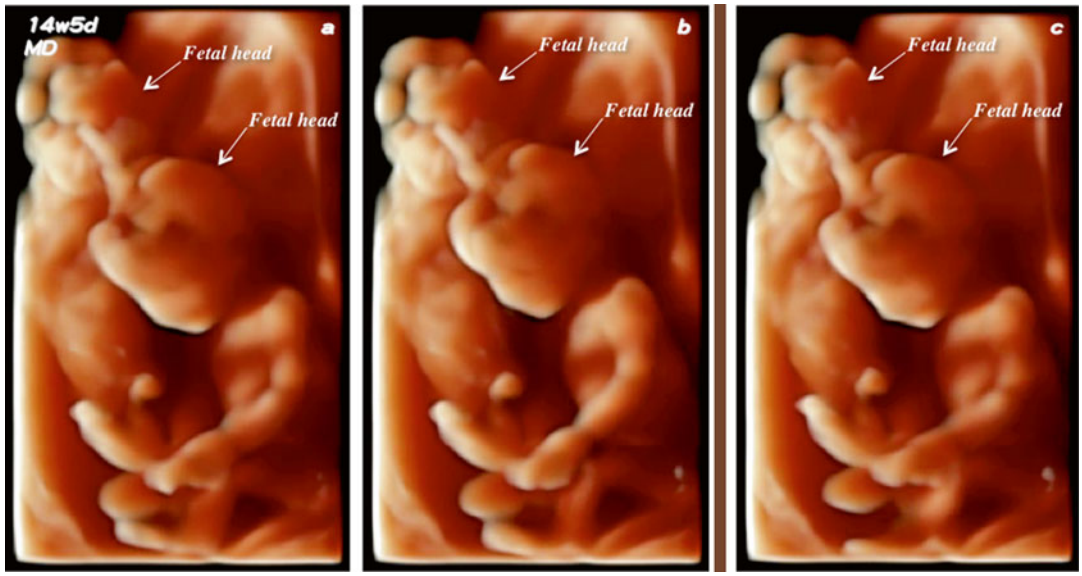

Fig. 19.9 Consecutive HDlive images of monochorionic diamniotic (MD) twin fetuses at 14 weeks and 5 days of gestation (**a**–**c**)

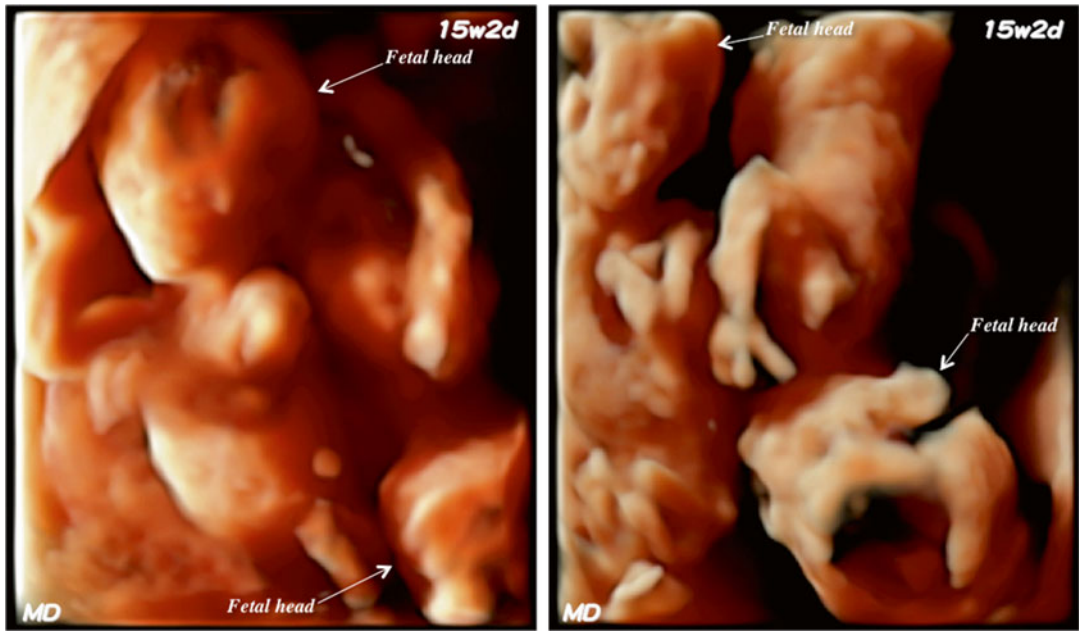

Fig. 19.10 HDlive images of monochorionic diamniotic (MD) twin fetuses at 15 weeks and 2 days of gestation. All anatomical whole-body structures can be recognized. One twin fetus looks like she is making a peace sign using her fingers

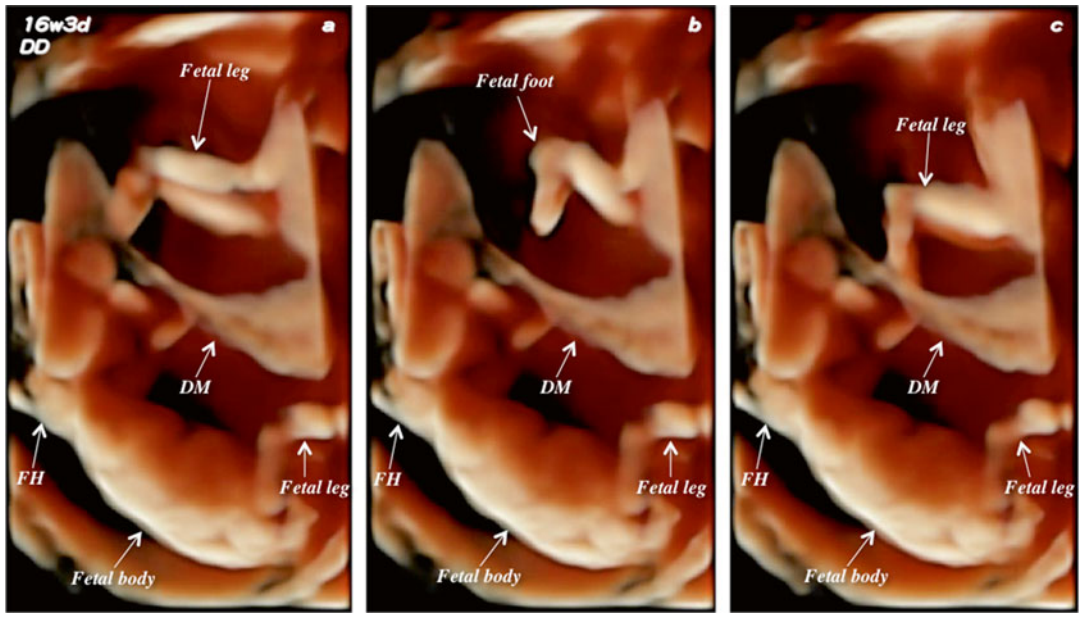

Fig. 19.11 Consecutive HDlive images of dichorionic diamniotic (DD) twin fetuses at 16 weeks and 3 days of gestation (**a**–**c**). Leg movements of one twin fetus are noted. *DM* dividing membrane, *FH* fetal head

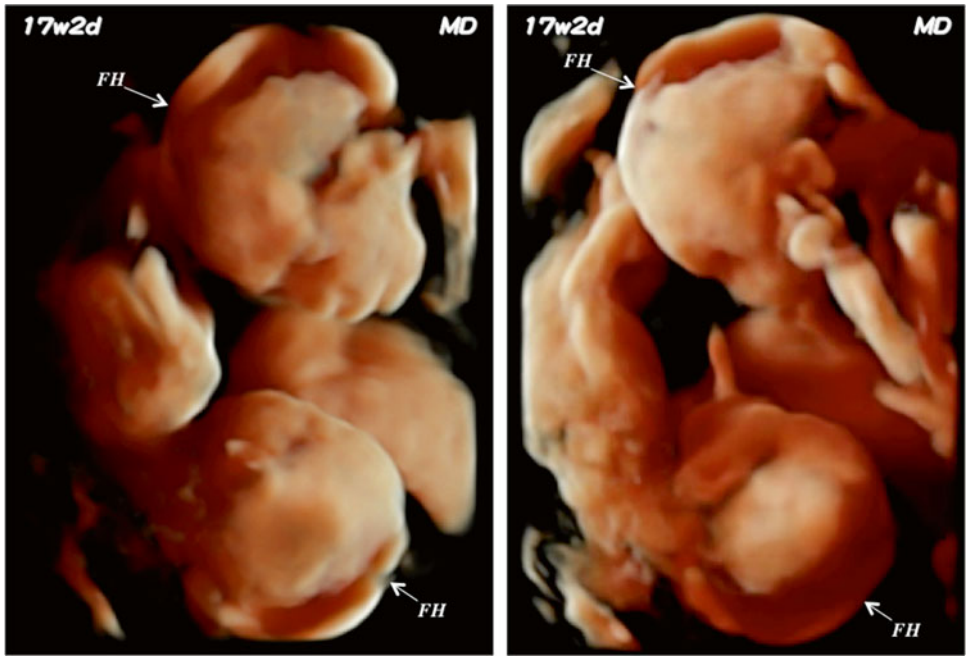

Fig. 19.12 HDlive images of monochorionic diamniotic (MD) twin fetuses at 17 weeks and 2 days of gestation. *FH* fetal head

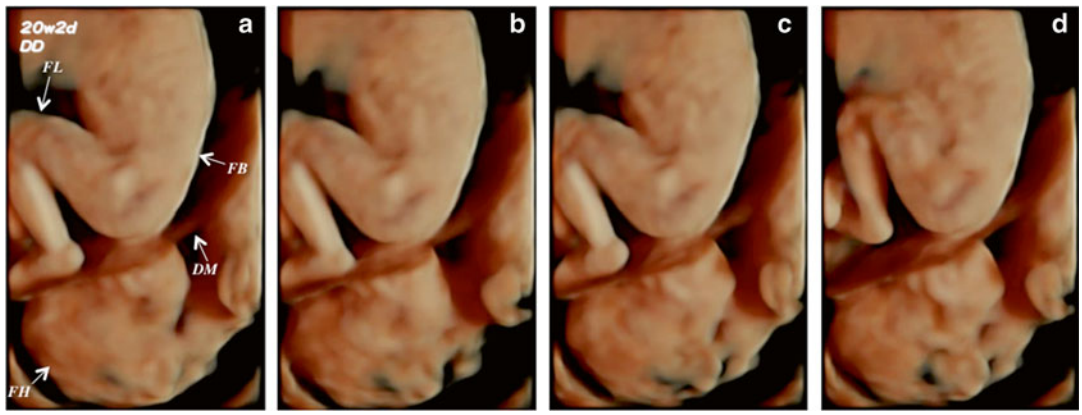

Fig. 19.13 Consecutive HDlive images of dichorionic diamniotic (DD) twin fetuses at 20 weeks and 2 days of gestation (**a**–**d**). The thin membrane (DM) between the two fetuses is noted. *FB* fetal body, *FH* fetal head, *FL* fetal leg

can be identified at this age. The frequencies of no reaction (twins appear to touch each other but there is no clear reaction) and reaction (twins appear to touch each other and there is a clear reaction by the co-twin) movements also are evaluated using 4D ultrasound at 12–13 weeks of gestation (Hata, Kanenishi, Sasaki, & Yanagihara, 2011). The median rate of reaction movements is 33.9 %. Hata, Kanenishi, Hanaoka, Sasaki, and Yanagihara (2012b) were the first to report on 4D sonographic assessment of inter-twin contact in a case of MD twins with acrania of one twin fetus late in the first trimester. They suggested that the decreased number of inter-twin contacts they

noted in the late first trimester might have been due to decreased frequencies of some movement patterns in the anencephalic fetus.

Several reaction movements after various types of contact can be clearly identified employing HDlive (illustrated in Figs. 19.15, 19.16, 19.17, 19.18, 19.19, 19.20, 19.21, and 19.22). Reaction movements (twins appear to touch each other and there is a clear reaction by the co-twin) usually occur within a few seconds. No reaction movement (twins appear to touch each other but there is no clear reaction) is also clearly evident (Figs. 19.21 and 19.22). In identifying inter-twin contact and intra-pair stimulation, the main advantage of an ultrasound device equipped with HDlive technology is that the light source can be freely positioned in order to illuminate the area of interest, allowing a better image quality, a better sensation of depth, and easier visualization of fetal surfaces (Bonilla-Musoles, Raga, Osborne et al. 2013). Hanaoka et al. (2014) reported that HDlive can provide clearer facial images than conventional three-dimensional (3D) ultrasound. In particular, HDlive is superior to conventional 3D ultrasound regarding the depiction of eye fissures because of its shadowing effect (Fig. 19.23). These researchers state that HDlive may be a useful diagnostic modality for the antenatal evaluation of subtle fetal facial dismorphism. In twin fetuses, we can clearly and easily recognize exact and precise inter-twin contacts using HDlive

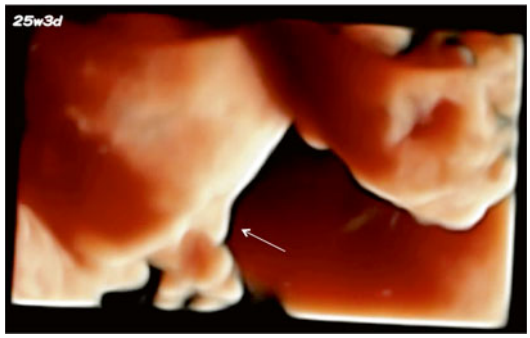

Fig. 19.14 HDlive image of twin fetuses at 25 weeks and 3 days of gestation. One twin fetus seems to be crying (*arrow*)

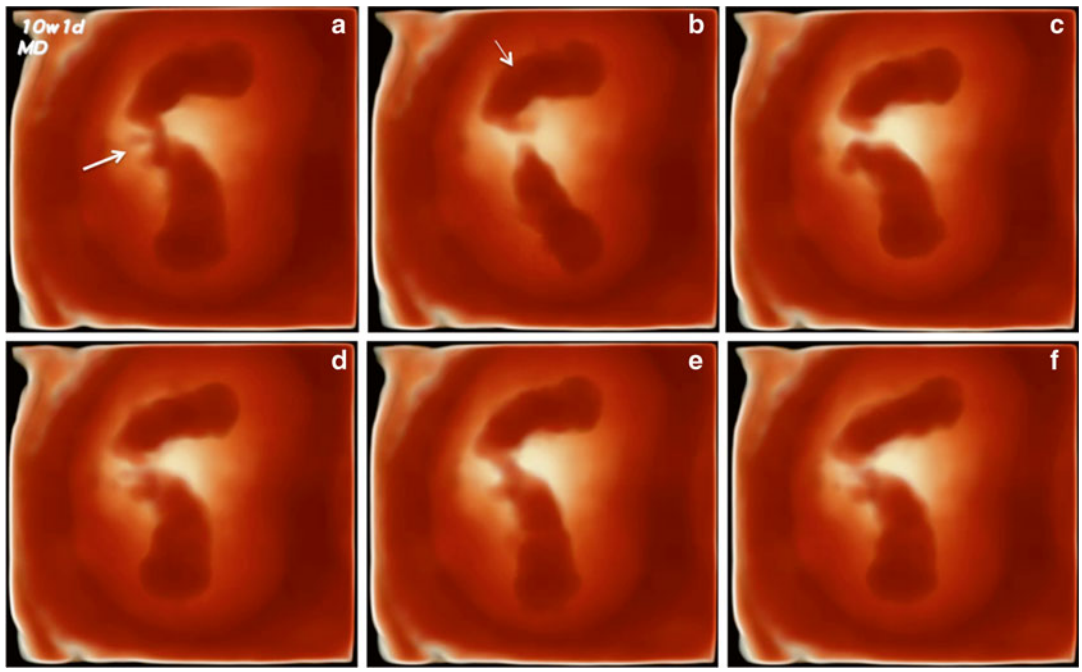

Fig. 19.15 Consecutive HDlive observations of monochorionic diamniotic (MD) twin fetuses at 10 weeks and 1 day of gestation (**a**–**f**). Leg-to-leg contact (*large arrow*) is clearly recognized, and there is a clear reaction of the co-twin fetus, who shows jumping movement (*small arrow*)

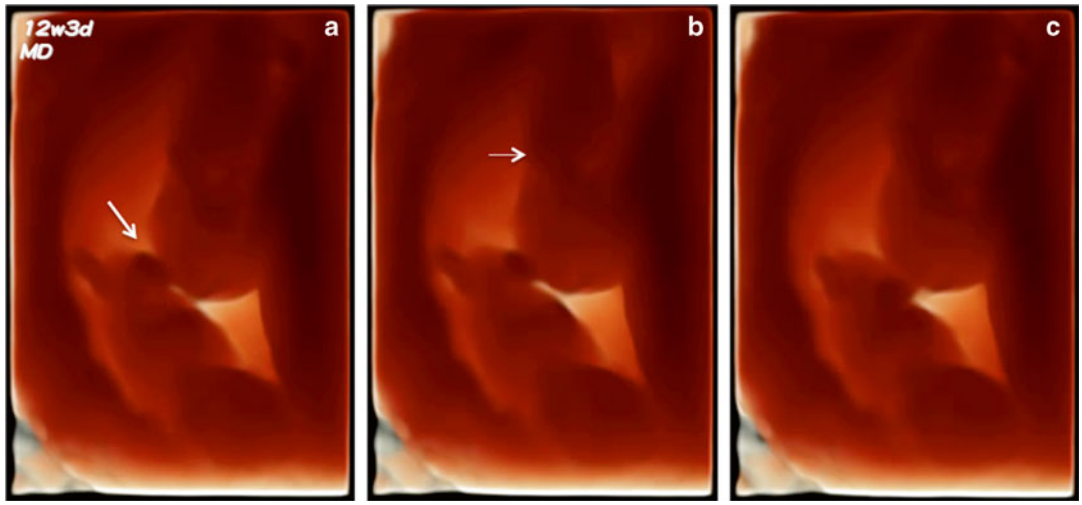

Fig. 19.16 Consecutive HDlive observations of monochorionic diamniotic (MD) twin fetuses at 12 weeks and 3 days of gestation (**a**–**c**). Leg-to-head contact (*large arrow*) is noted, and there is a clear reaction of the co-twin fetus (*small arrow*)

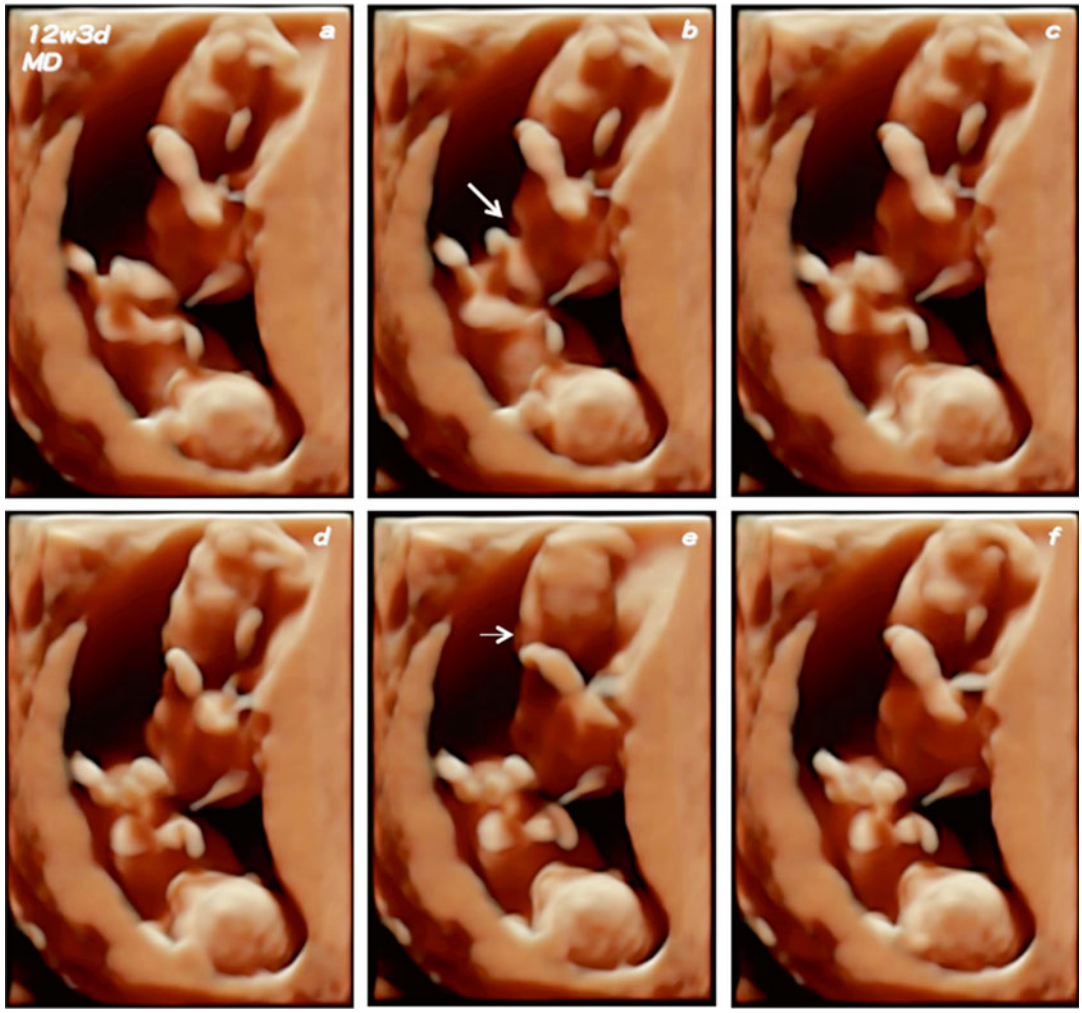

Fig. 19.17 Consecutive HDlive observations of monochorionic diamniotic (MD) twin fetuses at 12 weeks and 3 days of gestation (**a**–**f**). Leg-to-head contact (*large arrow*) is evident, and there is a clear reaction of the co-twin fetus (*small arrow*)

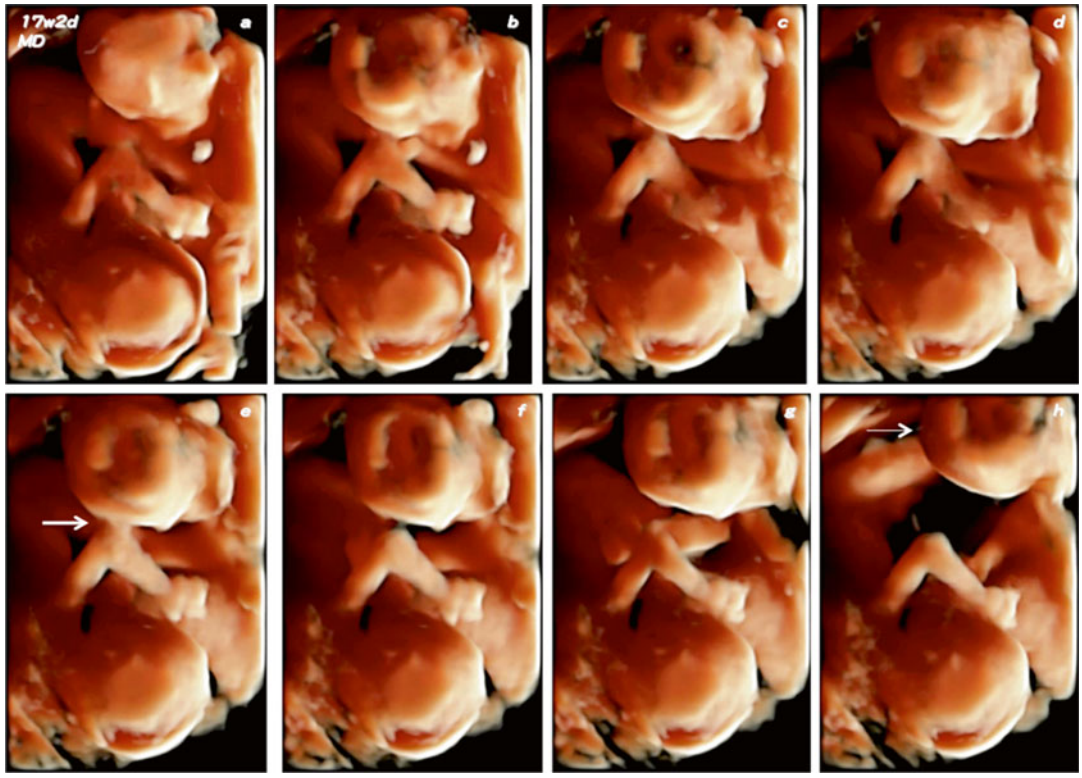

Fig. 19.18 Consecutive HDlive observations of monochorionic diamniotic (MD) twin fetuses at 17 weeks and 2 days of gestation (**a**–**h**). Arm-to-head contact (*large arrow*) is clearly noted, and there is a clear reaction of the co-twin fetus (*small arrow*)

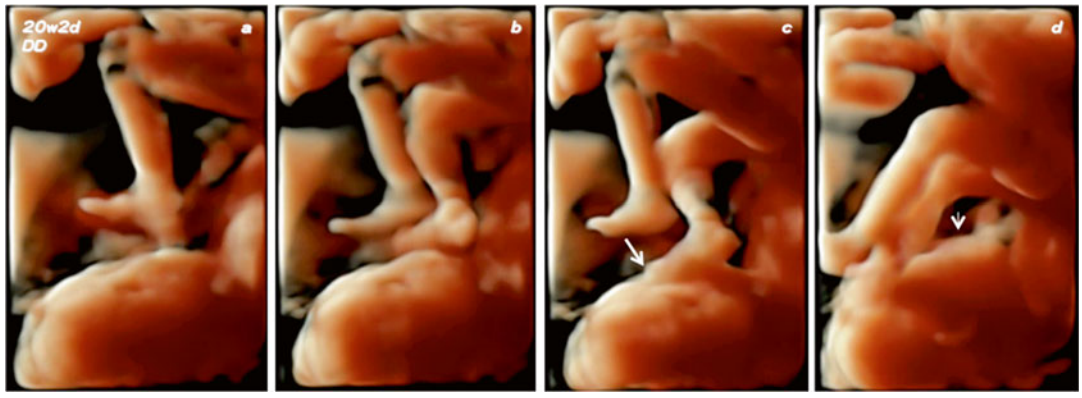

Fig. 19.19 Consecutive HDlive observations of dichorionic diamniotic (DD) twin fetuses at 20 weeks and 2 days of gestation (**a**–**d**). Leg-to-head contact (kick in the face) is clearly evident (*large arrow*), and there is a clear reaction of the co-twin fetus, who turns his face to the left (*small arrow*)

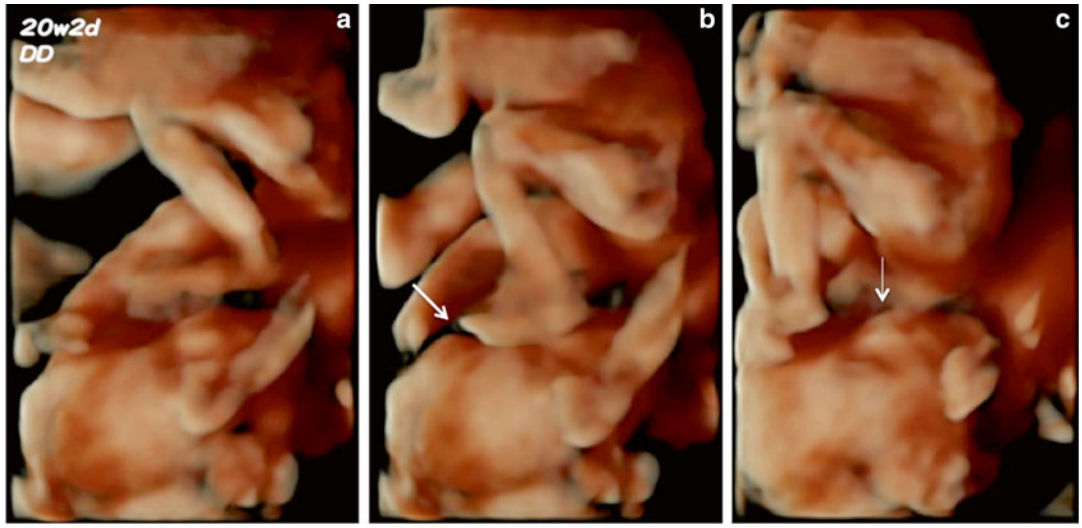

Fig. 19.20 Consecutive HDlive observations of dichorionic diamniotic (DD) twin fetuses at 20 weeks and 2 days of gestation (**a**–**c**). Leg-to-head contact (kick in the face) is clearly evident (*large arrow*), and there is a clear reaction of the co-twin fetus, who seems to show disappointment (*small arrow*) (**c**)

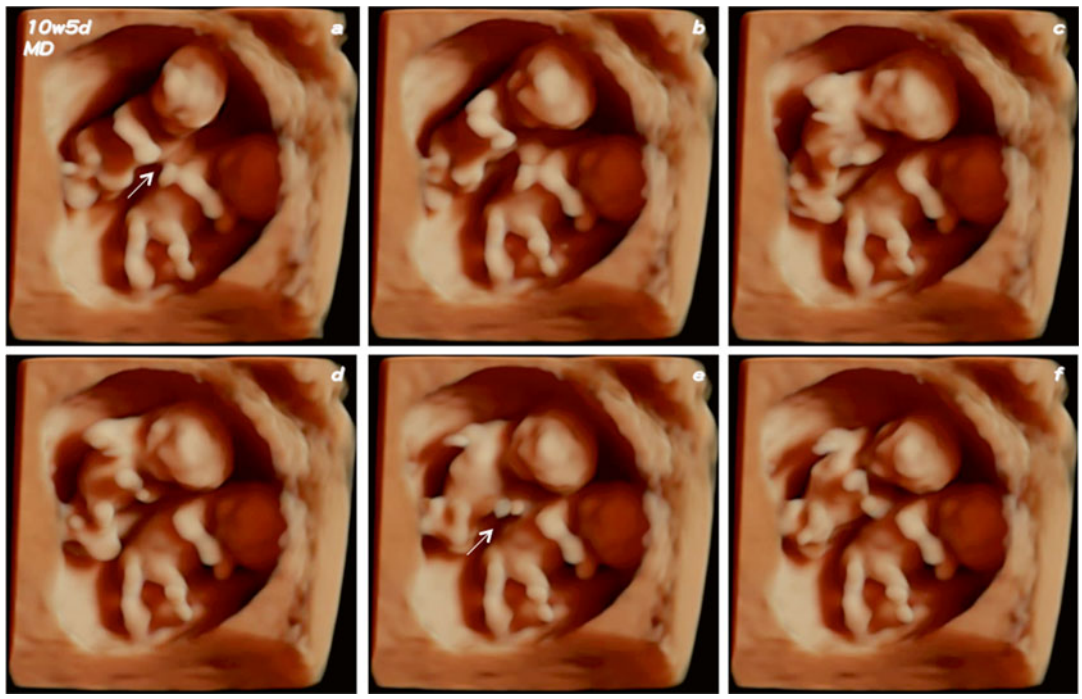

Fig. 19.21 Consecutive HDlive observations of monochorionic diamniotic (MD) twin fetuses at 10 weeks and 5 days of gestation (**a**–**f**). Repeated arm-to-trunk contacts (*arrows*) are evident. However, there is no clear reaction of the co-twin fetus

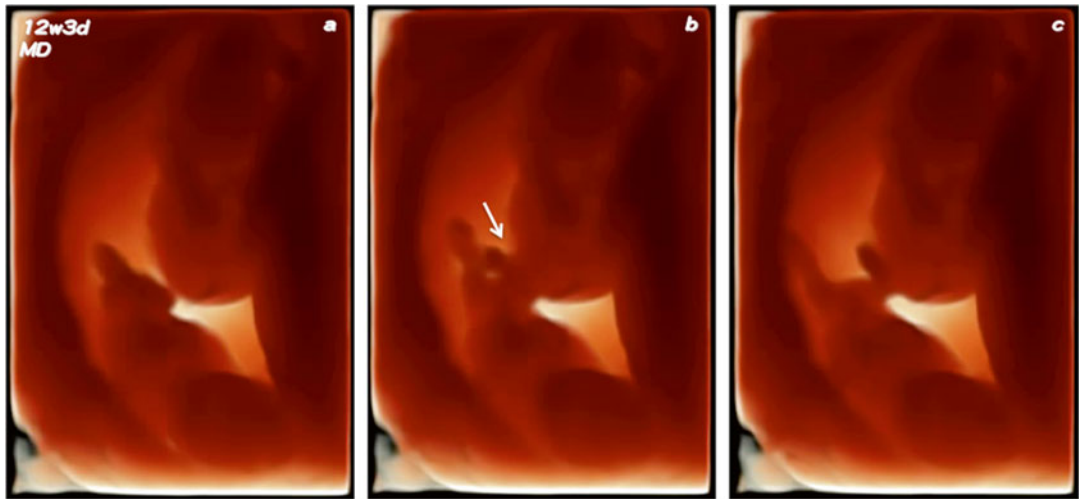

Fig. 19.22 Consecutive HDlive observations of monochorionic diamniotic (MD) twin fetuses at 12 weeks and 3 days of gestation (**a**–**c**). Leg-to-head contact (*arrow*) is noted. However, there is no clear reaction of the co-twin fetus

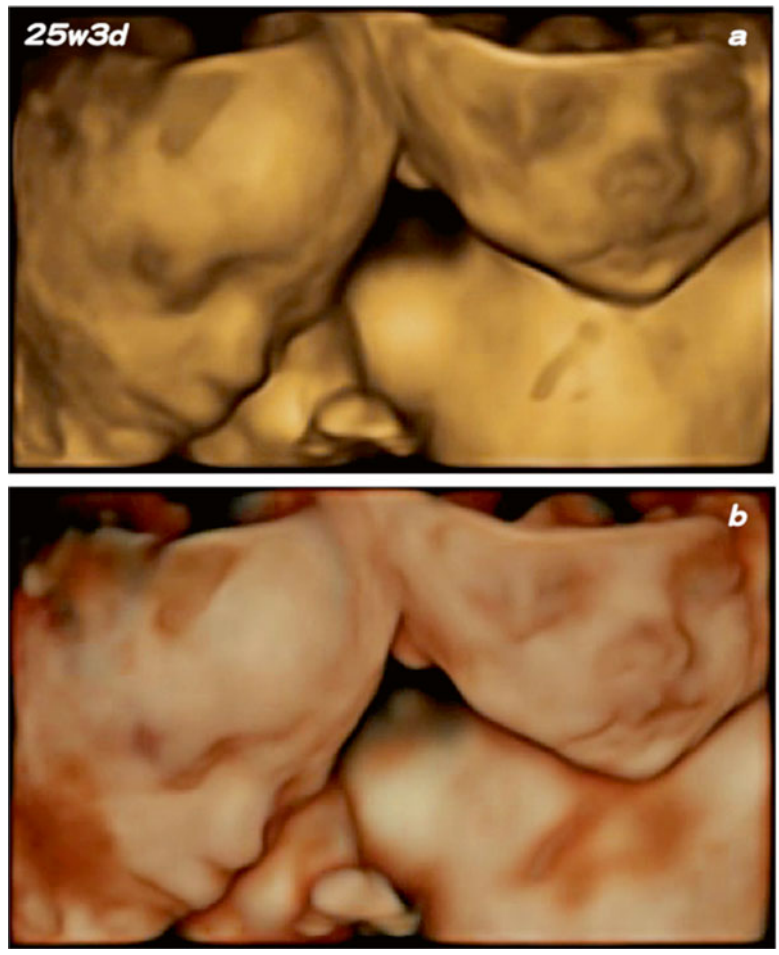

Fig. 19.23 Fetal faces in twin fetuses at 25 weeks and 3 days of gestation. The fetal facial anatomy depicted by HDlive seems to be much clearer compared to that by conventional four-dimensional (4D) ultrasound. (**a**) Conventional 4D sonographic image; (**b**) HDlive image

compared to conventional 4D ultrasound (Figs. 19.24, 19.25, and 19.26). These findings suggest that HDlive is an accurate and reliable modality for the assessment of fetal inter-twin contacts or intra-pair stimulations in twin pregnancies. HDlive may become an important technique in future behavioral research on twins.

Twin Activity Level and Later Temperament

Degani, Leibovitz, Shapiro, and Ohel (2009) studied inter-twin differences in activity using conventional 4D ultrasound at 11–14 weeks of gestation, and examined their relationship with infant twins' subsequent temperament. The more active twin in each pair showed a close correlation with prenatal inter-twin differences in activity. These researchers suggest that differences in activity in each pregnancy, even before the emergence of fetal behavioral patterns, are followed by differences in the temperament activity level postnatally. Honemeyer and Kurjak (2012) applied the Kurjak Antenatal Neurodevelopmental Test (KANET) assessed by conventional 4D ultrasound at different gestational ages to both fetuses of a DD twin pregnancy, and compared the results with temperament evaluation of both neonates at 8 weeks postpartum. They suggest that temperament is primarily controlled by constitutional factors, and the modulation of these primary settings may be caused by superimposed environmental factors later on. If we use HDlive to assess each twin fetal movement, we may be able to identify differences in the frequency and activity of twin fetuses in the late first and second trimesters (Figs. 19.27, 19.28, 19.29, 19.30, and 19.31). The application of HDlive to assess fetal movement in twins may improve the usefulness and reproducibility of the KANET scoring system

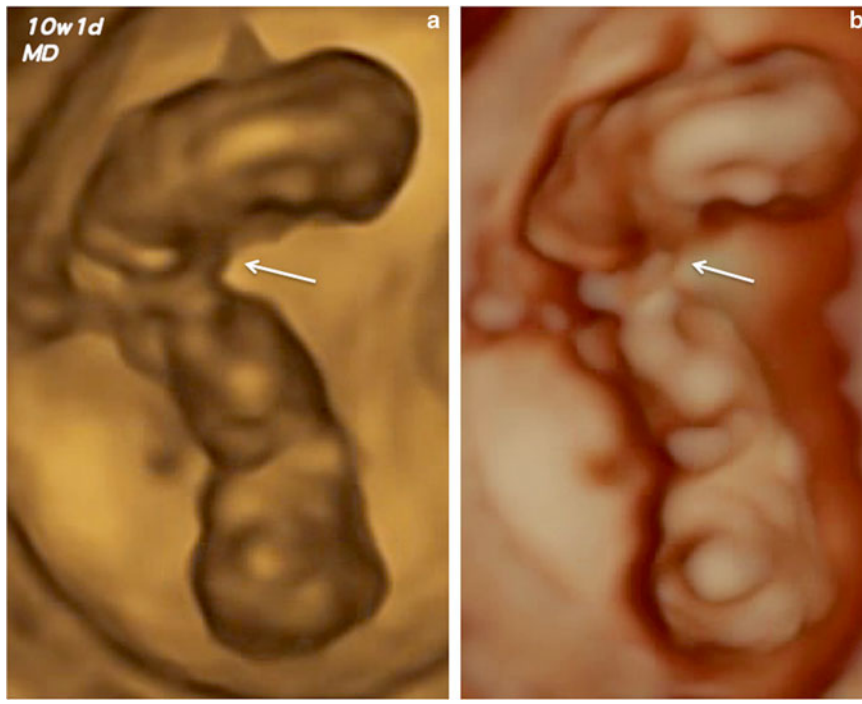

Fig. 19.24 A monochorionic diamniotic (MD) twin pregnancy at 10 weeks and 1 day of gestation. HDlive image of leg-to-trunk contact (*arrows*) seems to be more readily discernible than that obtained by conventional four-dimensional (4D) ultrasound. (**a**) Conventional 4D sonographic image; (**b**) HDlive image

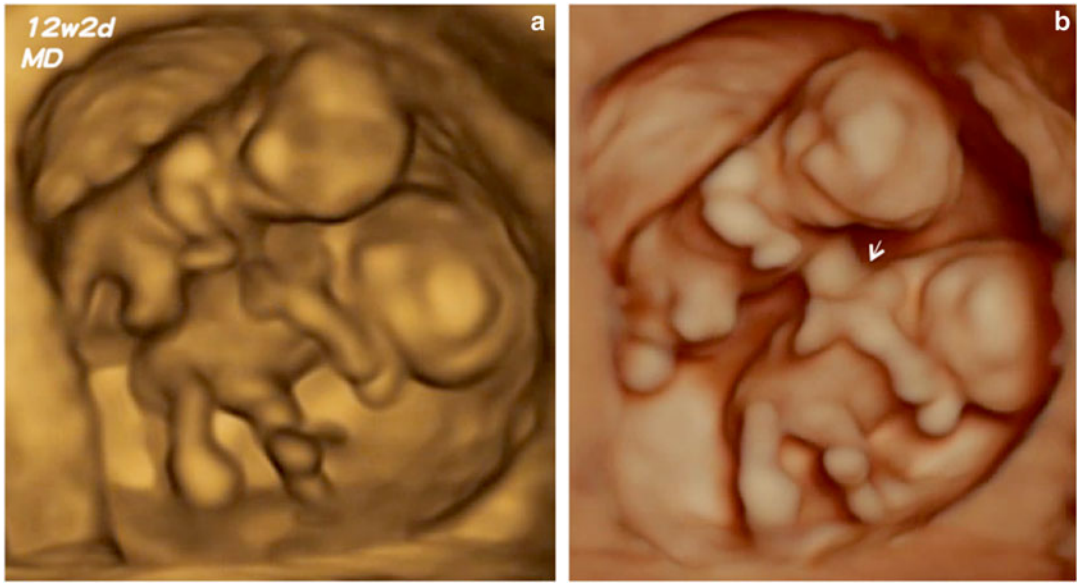

Fig. 19.25 A monochorionic diamniotic (MD) twin pregnancy at 12 weeks and 2 days of gestation. The HDlive image of arm-to-trunk contact (*arrow*) seems to be more readily discernible than that obtained by conventional four-dimensional (4D) ultrasound. (**a**) conventional 4D sonographic image; (**b**) HDlive image

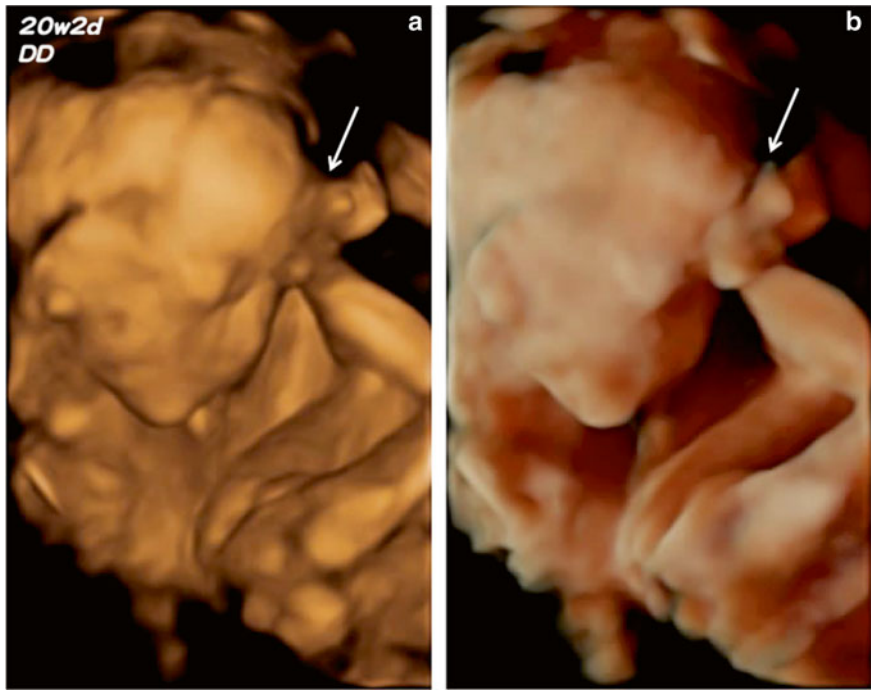

Fig. 19.26 A dichorionic diamniotic (DD) twin pregnancy at 20 weeks and 2 days of gestation. The HDlive image of leg-to-face contact (*arrows*) seems to be more readily discernible than that obtained by conventional four-dimensional (4D) ultrasound. (**a**) Conventional 4D sonographic image; (**b**) HDlive image

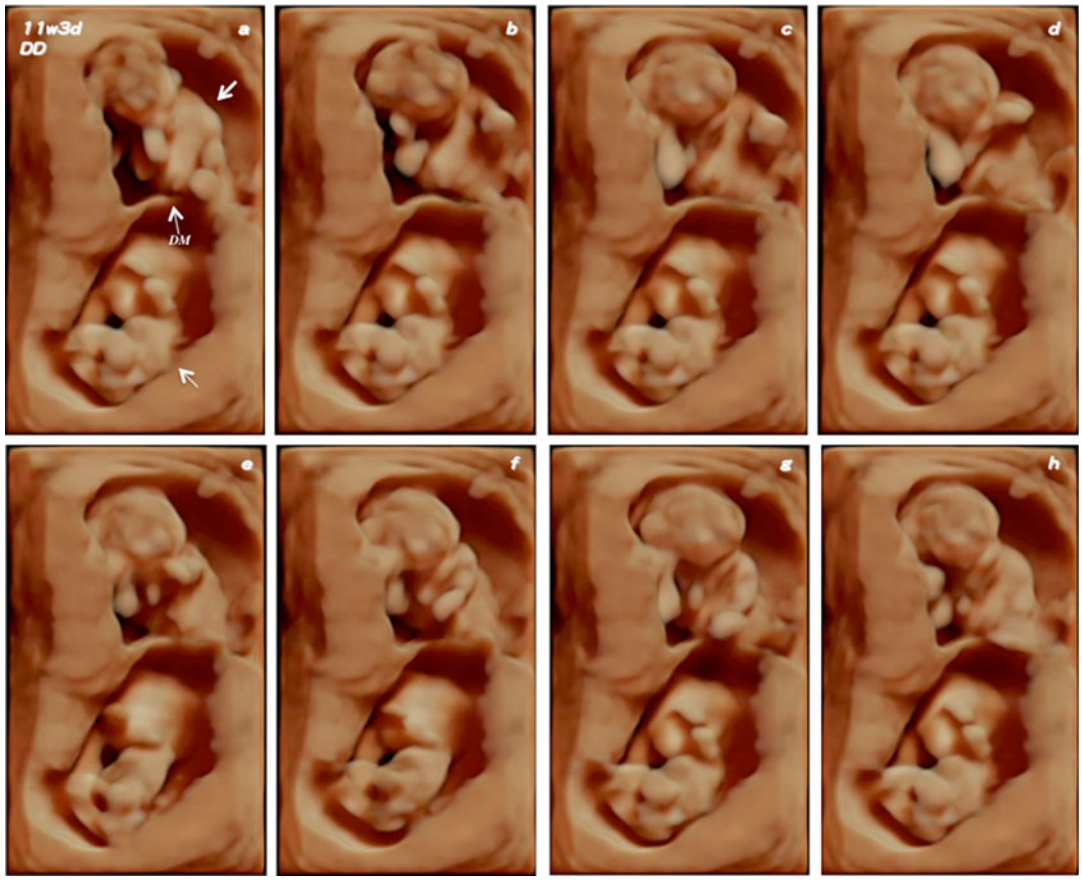

Fig. 19.27 Consecutive HDlive observations of dichorionic diamniotic (DD) twin fetuses at 11 weeks and 3 days of gestation (**a**–**h**). Active general movements of one twin fetus are evident (*large arrow*), but there is no movement of the co-twin fetus (*small arrow*). *DM* dividing membrane

Limitations of HDlive

Limitations in optimal visualization of the surface fetal structures in twins using HDlive occur in cases of inappropriate fetal positions or with the fetal structures of interest adjacent to the placenta or uterine wall (Hanaoka et al., 2014). These limitations are the same as with conventional 4D ultrasound. Moreover, mastering this rendering mode involves a learning curve, and not all physicians and sonographers may be familiar with it.

Conclusions

The software and a movable virtual adjustable light source in HDlive can be used to calculate light propagation through surface structures

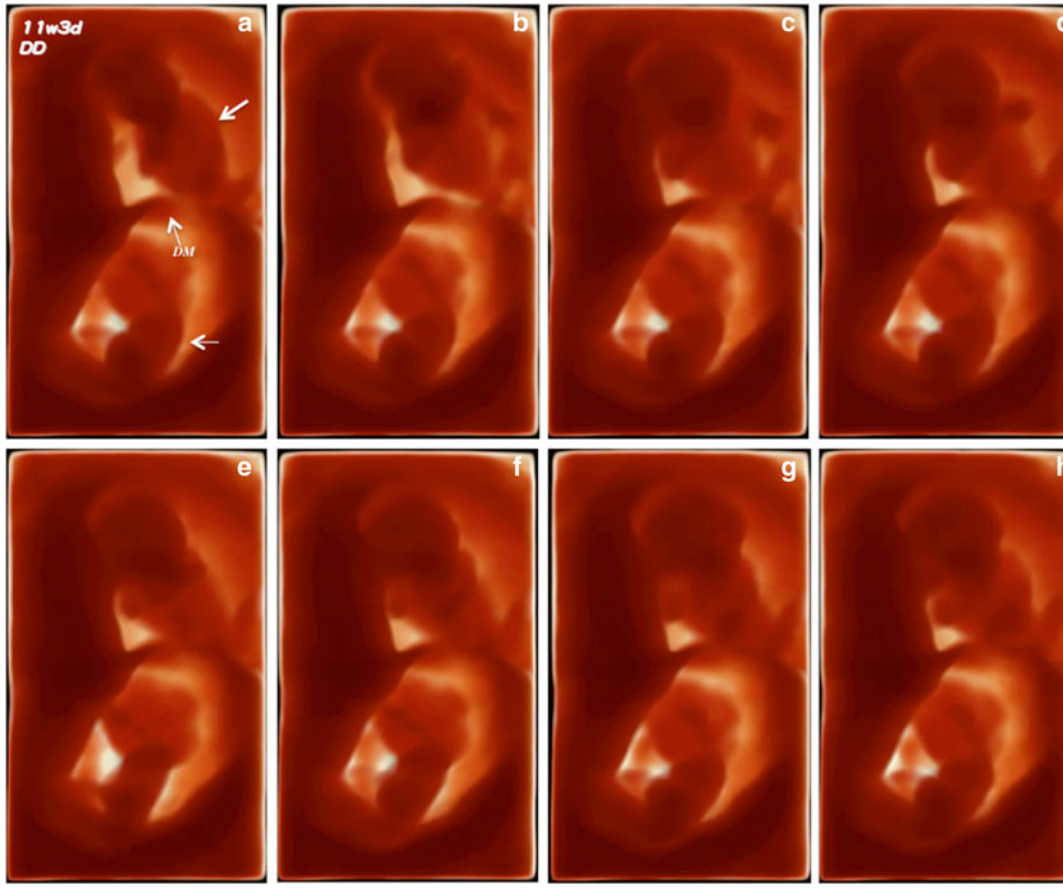

Fig. 19.28 Consecutive HDlive observations of dichorionic diamniotic (DD) twin fetuses at 11 weeks and 3 days of gestation (**a**–**h**). Active general movements of one twin fetus are evident (*large arrow*), but there is no movement of the co-twin fetus (*small arrow*). *DM* dividing membrane

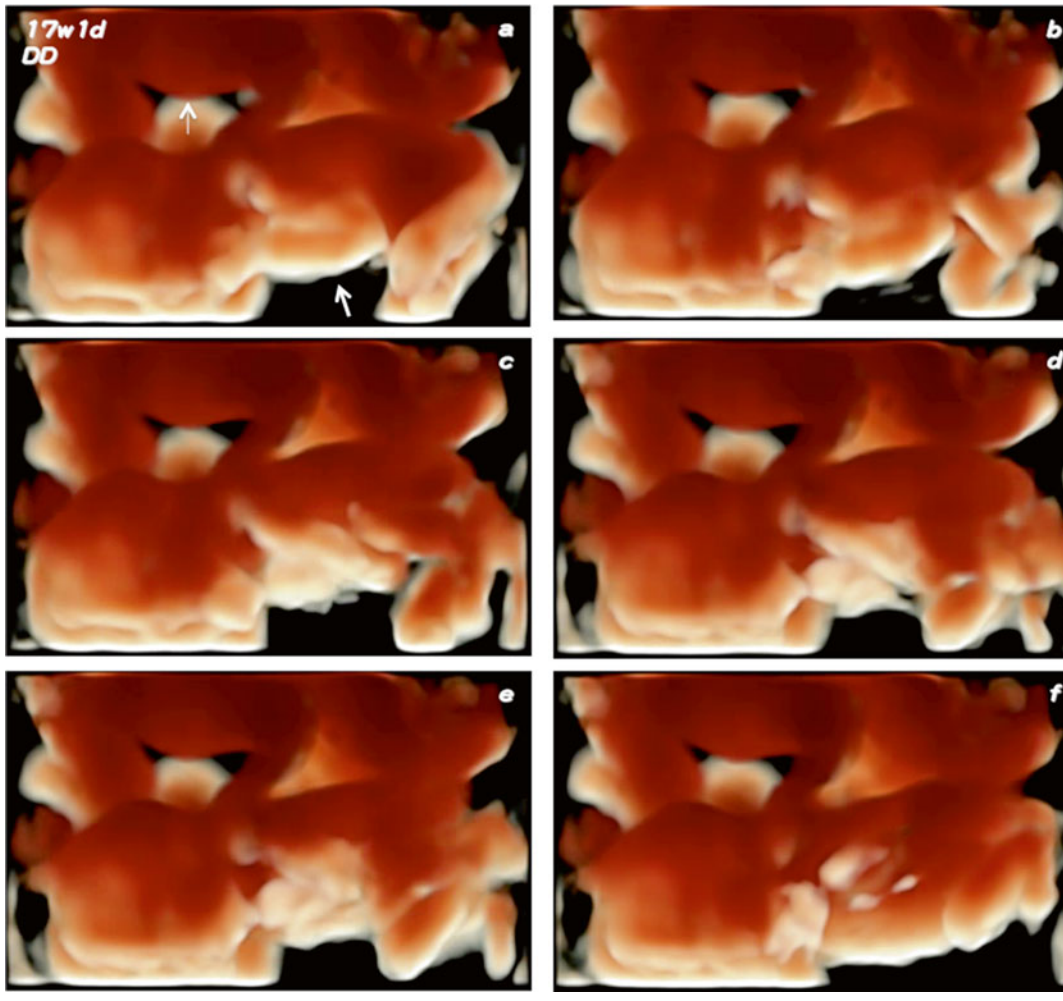

Fig. 19.29 Consecutive HDlive observations of dichorionic diamniotic (DD) twin fetuses at 17 weeks and 1 day of gestation (**a**–**f**). Active general movements of one twin fetus are evident (*large arrow*), but there is no movement of the co-twin fetus (*small arrow*)

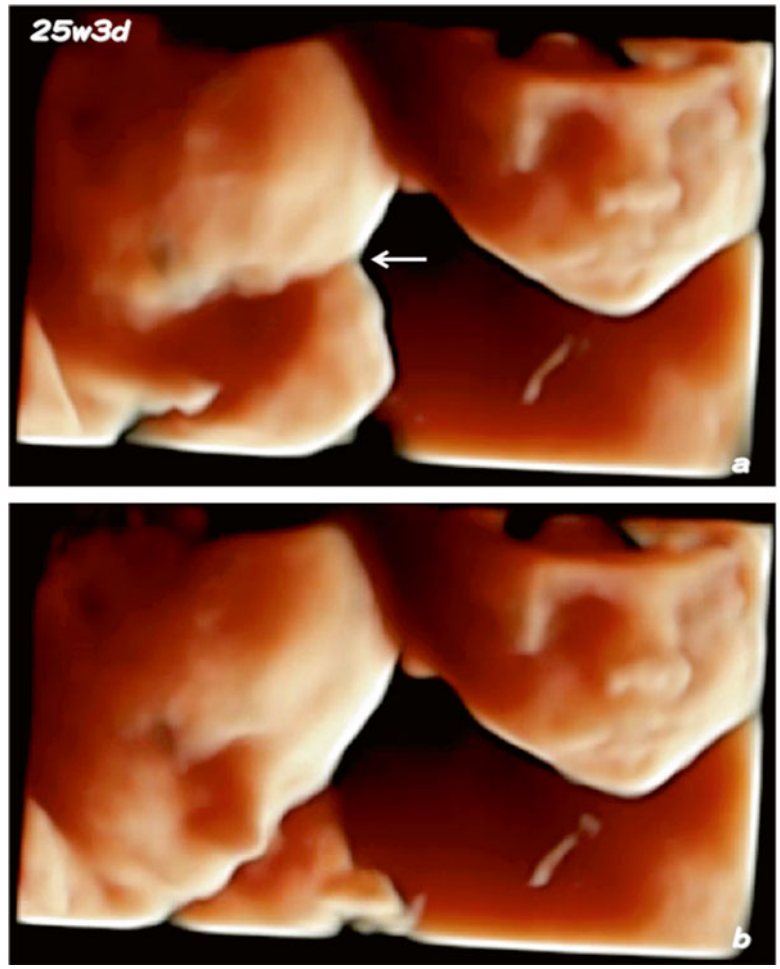

Fig. 19.30 Consecutive HDlive observations of twin fetuses at 25 weeks and 3 days of gestation (**a**, **b**). Hand-to-face movement of one twin fetus is evident (*arrow*), but there is no movement of the co-twin fetus

based on the direction of light. An anatomically realistic image with natural skin-like colors of the normal and abnormal embryos and fetuses is possible with this ultrasound software (Kagan et al., 2011; Merz, 2012). The use of HDlive facilitated natural, realistic images of both embryos and fetuses in MD and DD pregnancies, with the images being much more detailed compared to those of conventional 3D/4D ultrasound. In all three trimesters of pregnancy, HDlive generated more realistic images to assess twin pregnancy; thus, it may become a very important modality for twin research and the antenatal evaluation of abnormal twin pregnancies during pregnancy in the future.

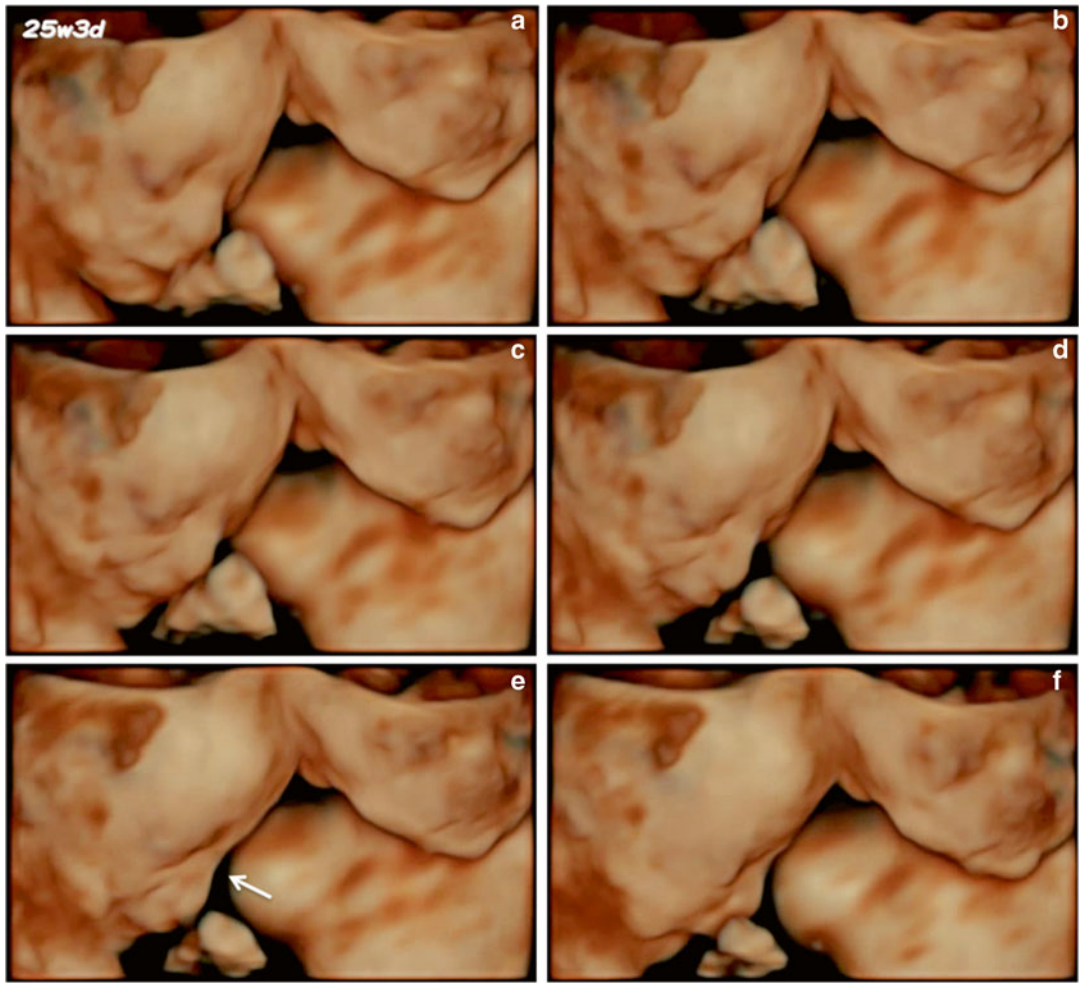

Fig. 19.31 Consecutive HDlive observations of twin fetuses at 25 weeks and 3 days of gestation (**a**–**f**). Hand-to-face movement of one twin fetus, who seems to be crying (*arrow*), is clearly noted, but there is no movement of the co-twin fetus

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Conflict of interest: The authors declare no conflict of interest.

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In Vivo Human Fetal Brain Analysis Using MR Imaging

20

François Rousseau, Colin Studholme, Renaud Jardri, and Moriah E. Thomason

Abstract

This chapter provides a review of the current research into in utero magnetic resonance (MR) imaging of the human fetal brain. It is divided into three parts: structural imaging, diffusion imaging, and functional imaging. In each part, a description of MR sequences is provided, as well as advanced image processing techniques that are used to build 3D images from motion scattered slices. Combination of fast MR techniques and post-processing algorithms leads to key applications in the context of in utero fetal brain studies such as brain folding analysis, connectome analysis, and functional network analysis. The recent advances in in utero fetal brain MR imaging described in this chapter open new perspectives in early brain development understanding.

Keywords

Human fetal brain • MRI • Functional MRI • Diffusion MRI • Motion compensation • Image reconstruction • Connectome • Functional connectivity

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Introduction

Ultrasound (US) imaging is widely used in clinical practice for visualizing and monitoring fetal development. Advantages of US are real time capability, high-resolution images, long history in safety and equipment availability. However, US imaging for fetal brain study is still limited by the presence of the skull and its ability to distinguish subtle differences in brain tissues. It rapidly appeared that Magnetic Resonance Imaging (MRI) could provide detailed in utero fetal images allowing for the study of the process of brain tissue growth. Compared to US, MRI allows for the use of other acquisition procedures such as functional MRI, diffusion, and MR spectroscopy (de Vries, 2006).

MRI, however, has several limitations for fetal brain study due to acquisition time constraints. Owing to relatively low signal strength, fetal brain MRI examination requires relatively long imaging times, which increases susceptibility to unavoidable fetal movements during data acquisition. Although the use of fast sequences has improved the feasibility of in vivo fetal brain MRI (Baert & Prayer, 2011), data acquisition remains performed in a slice-by-slice fashion. This has led to the acquisition of detailed brain tissue 2D images (with a resolution less than 1 mm). 3D quantitative information extracted for MR stacks was, until the recent development of new dedicated image processing techniques, not easily accessible. This chapter reviews some recent advances in fetal brain MR imaging, focusing on structural, diffusion, and functional MRI and the related data processing approaches.

Structural Imaging

Sequences

The first MR images of the fetal brain were obtained more than 30 years ago (Smith, Adam, & Phillips, 1983). At that time T1- and proton density-weighted acquisitions were performed in six patients to measure characteristics of the brain in utero (i.e., fetal biparietal diameter, crown–rump length, trunk circumference, area). They acquired data using a very low-field MRI unit (less than 0.1 T). In this pioneering work, the authors noted the potential of this imaging modality for providing a detailed visualization of brain anatomy in the living human fetal brain. They recognized MRI as a promising method for analysis of development and growth of the human fetus. As such, MRI emerged as a suitable complement to ultrasound, providing improved visualization of the fetal brain. However, MRI examination remained confined to research because acquisition time was not compatible with application in routine clinical settings.

The 1990s witnessed significant improvements in MR acquisition sequences [such as EPI (Mansfield et al., 1990), HASTE (Yamashita et al., 1997) and SSFSE (Busse, Riederer, Fletcher, Bharucha, & Brandt, 2000)]. These sequences have enabled rapid acquisition of individual slices (2D image) in less than a second (more details on how to shorten MRI sequences can be found for instance in Baert & Prayer, 2011). Thus, a minute or less became sufficient to acquire the whole fetal brain. Fast acquisition sequences have allowed introduction of the MR imaging modality in clinical settings for in vivo fetal analysis as a complement to ultrasound. In this context, the use of fetal MRI has greatly expanded.

During this period, the MR sequences used for in utero visualization diversified, leading to the creation of several fetal MRI atlases (see for instance Garel, 2004 and Levine, 2005). These atlas books highlight the importance of having a reference for interpreting these new images, which remain inherently 2D. Indeed, the atlas books give a better understanding and interpretation of the 3D brain geometry from the 2D acquisitions.

However, as pointed out by Levine (2001) in an article on the future of fetal MRI, despite the decrease in acquisition time, 3D brain in utero study remains difficult because of fetal movement. Due to movements occurring during the acquisition, fetal brain MRI data remain acquired in a slice-by-slice fashion, not as a full 3D acquisition. This slice-based approach is less sensitive to motion artifacts of the subject (ghosting and blurring) but it does not allow 3D quantitative studies

of the fetal brain. In addition, the approaches used to correct the movement of the subject in adults (both retrospective or prospective motion correction) are not suited to the study of the brain in utero (for further details, see Studholme, 2011).

Motion Correction

Nowadays, there is a new research trend to reach full 3D fetal brain MRI by using advanced image post-processing techniques combined with fast snapshot multi-slice imaging. The starting point of such research is that several complementary multi-slice acquisitions in different spatial directions (axial, coronal, and sagittal) are acquired. These provide the radiologist a pseudo-3D visualization of the fetal brain. However, each slice location may still be corrupted by fetal or maternal motion occurring during data acquisition. As a result, it is necessary to estimate the motion (modeled by three translations and three rotations) to correct the positioning of each slice, to be able to estimate a spatially correct 3D image of the fetal brain. Each estimated rigid transformation map shifts slices into a consistent 3D coordinate system. The main issue compared to other motion correction problems in medical imaging is that the reference 3D volume is unknown, we simply have a collection of slices to stitch together.

The first approach developed for between slice motion correction of fetal brain MRI (Rousseau et al., 2005, 2006) was an iterative two-step "reconstruction based" technique. First, a 3D image was estimated from scattered MR measurements based on initial estimates for the rigid transformations for each slice. Second, these slice transformation estimations were refined relative to their match to the estimated reference 3D image. These two steps were then repeated to refine slice alignment. This iterative procedure was motivated by the classical linear formulation used in inverse problems (Kaipio & Somersalo, 2006) where the observations (the set of acquired MR 2D slices) are linked to the variable to estimate (the 3D fetal brain MR image) with a linear observation operator modeling fetal motion, intensity variations, subsampling and the point spread function (PSF) parameters. In such a context, the unknowns are estimated using the iterated conditional modes algorithm (Besag, 1986).

However, in this initial reconstruction based framework, the problem is addressed iteratively in two-stage fashion where the fitting criteria for the 3D image reconstruction step (estimating voxel values on a regular grid) is not identical to that being optimized in the slice alignment step. This leads to a lack of any guarantee of overall convergence of the alternating steps, particularly when there are regions of missing slices due to motion, which may induce blurring in the 3D reconstruction step. One approach to overcoming this issue is to completely decouple the slice alignment process and the final 3D reconstruction. To do this Kim et al. (2010, 2008) developed a direct slice alignment approach that minimizes the discrepancy in intensities along slice intersections, rather than their match difference to a putative 3D reconstruction estimate. After slice alignment, the final direct slice transformation estimates for the motion scattered slices can then be used to reconstruct a 3D image on a regular voxel lattice. For cases of smaller numbers of acquired slices, this technique also has the computational advantage of not requiring the reconstruction of a 3D image during the iterative motion estimation process.

The iterative 3D reconstruction-based approach also has been refined by several groups in different ways to make better use of the high resolution provided within the 2D slice plane to form 3D images with finer 3D isotropic resolution. All early reconstruction algorithms made use of the same linear observation model typically considered in inverse problems to essentially interpolate scattered slice measurements onto a regular lattice. Adding a PSF model within a deconvolution framework, particularly in the through-plane direction, allows a finer image reconstruction when the anatomy is oversampled from many directions, and the estimated 3D image can reveal more precise anatomical detail. Such a deconvolution based reconstruction process using a super-resolution framework has been described (Gholipour, Estroff, &

Warfield, 2010; Rousseau, Kim, Studholme, Koob, & Dietemann, 2010).

Slice intensity differences occurring due to spin history or motion modulated receiver coil bias field effects mean that the same anatomy imaged in different slice views exhibits different intensity levels. These induce artifacts in the final reconstruction and can also perturb the accuracy of the slice alignment process. These issues were first addressed in the early work of Rousseau et al. (2006) using a filtering-based 3D bias correction scheme relying on low frequency invariance. Later a direct approach using least square fitting of parametric models of intensity inconsistencies between individual slice data was developed (Kim et al., 2011), using the slice intersection framework, combined with a simple outlier rejection to address complete signal drop out due to severe spin history corruption of the intensities. Using a reconstruction based framework for slice alignment, a dedicated intensity outlier detection scheme was developed by Kuklisova-Murgasova, Quaghebeur, Rutherford, Hajnal, and Schnabel (2012).

Building on the improved iterative model based volume reconstruction methods, most recently, advances have led to unified model based approaches for slice alignment, which estimate slice transformations and a model based 3D reconstruction simultaneously (Fogtmann, Seshamani, Kim, Chapman, & Studholme, 2012). This is achieved by optimizing a single criterion (reflecting observed data consistency with a model) in a single energy minimization process. This provides a numerically more consistent framework for the 3D image formation process. Figure 20.1 shows an example of high-resolution fetal brain image reconstruction using super resolution framework.

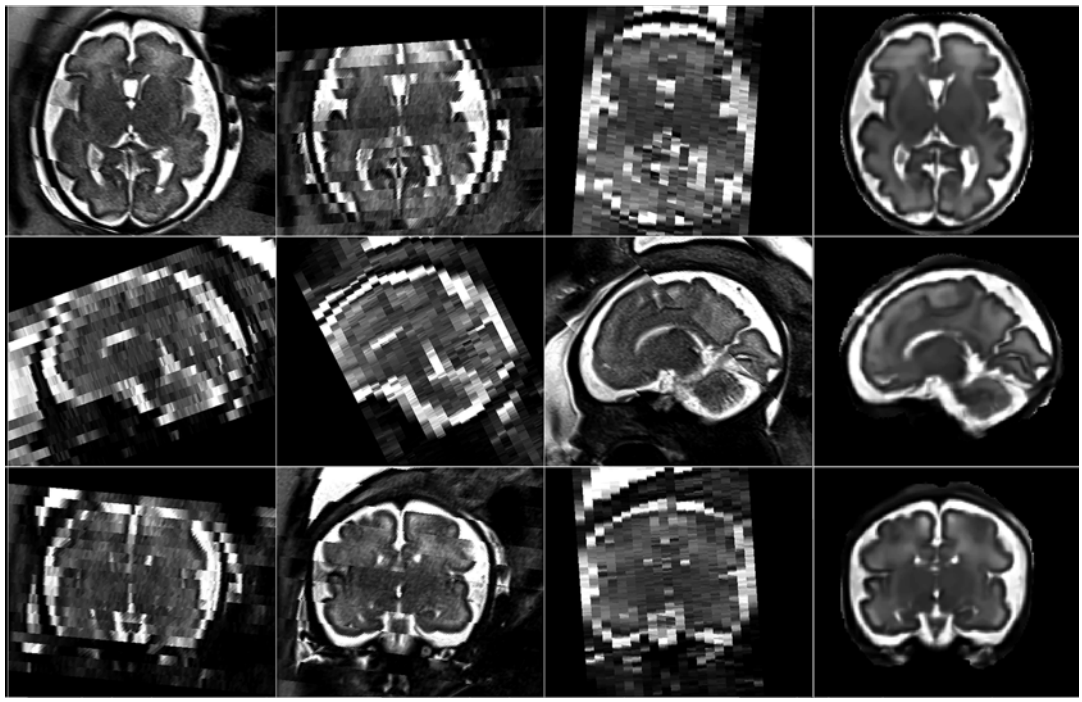

Fig. 20.1 Based on three orthogonal acquisitions (the *first three columns*), a 3D fetal brain MR image (*fourth column*) can be estimated

Segmentation

The human brain is a complex organ, which consists not only of different types of tissue but also a large number of structures whose size, shape, and function vary. Once motion and intensity corrections are performed, the first challenge in the analysis of MR images of the fetal brain consists of extracting structures of interest while minimizing errors in terms of false positives (false detections) and false negatives (omission error). Segmentation methods are mainly based on the observed MRI signal intensity with shape priors that are intended to delineate the various tissues according to their signal (value, texture) in the MR image. Many methods have been proposed for the analysis of T1-weighted MR images in adults. However, in fetal MR imaging, T2-weighted images are typically used to visualize brain anatomy. In addition to noise, intensity nonuniformity and partial volume effect, also present in adult brain MRI, are present in fetal studies to a greater extent because of the distance between the anatomy of interest and the receiver coils, and the relatively smaller anatomy being studied. Most importantly, the fetal brain at early to mid gestational ages consists of fundamentally different tissue regions to those seen in neonates, that do not directly correspond to the traditional gray and white matter of the developed neonatal or late gestation fetal brain. These transient zones include the germinal matrix (ventricular and subventricular zones), subplate (SP), intermediate zone (IZ), and cortical plate (CP, Kostovic & Judas, 2002).

One of the first automated segmentation methods dedicated specifically to fetal brain MRI explored approaches that extended traditional atlas driven tissue segmentation with temporally varying priors for these transient tissue zones (Habas et al., 2010). This four-dimensional parametric atlas was built from carefully, manually delineated MRI scans to provide an accurate statistical reference for a given age of development. Intensity of each brain structure to segment was modeled with a mixture of Gaussian distributions whose parameters were modeled over time by a polynomial that could capture the appearance and disappearance of tissue classes over time. This was successfully employed to study both normal fetal growth (Scott et al., 2011) and to accurately segment the cortical plate in cases of abnormal growth seen in isolated mild ventriculomegaly (Scott et al., 2012). An alternative to a fully parameterized 4D atlas has been investigated by making use of a multi-atlas strategy for the study of more severe forms of fetal ventriculomegaly (Gholipour, Akhondi-Asl, Estroff, & Warfield, 2012).

Fetal brain structures have many characteristics (i.e., variability in shape and intensity for instance) that may require the integration of prior knowledge in order to develop robust segmentation techniques. The use of a probabilistic atlas (as mentioned earlier) is one of the possibilities to introduce such prior knowledge that can drive the segmentation process toward an admissible solution. However, such techniques may introduce some bias when dealing with pathological data. It also may not be enough when strong priors are required, such as for instance cortical segmentation. In this particular case, knowledge about cortical thickness may be required to provide topologically correct segmentation maps (Caldairou et al., 2011). Figure 20.2 shows an example of fetal brain segmentation results.

Brain Growth Modeling

Fetal motion correction techniques in conjunction with segmentation methods have opened a route to study brain growth based on in utero MR data. One of the challenges of fetal MR image analysis concerns the understanding of fast anatomical changes occurring during this short period of time. This includes modifications of size and shape related to the brain growth, and changes in appearance (intensity of the white matter and gray matter, disappearance of the germinal matrix, etc.) related to the brain maturation process. In the context of age-specific atlas building, it appears that the longitudinal fetal data acquisition is ethically complicated. An age-specific atlas has then to be estimated from a set of heterogeneous data from different subjects, acquired at different ages. To do this, all images are mapped nonlinearly into a common reference space in which a

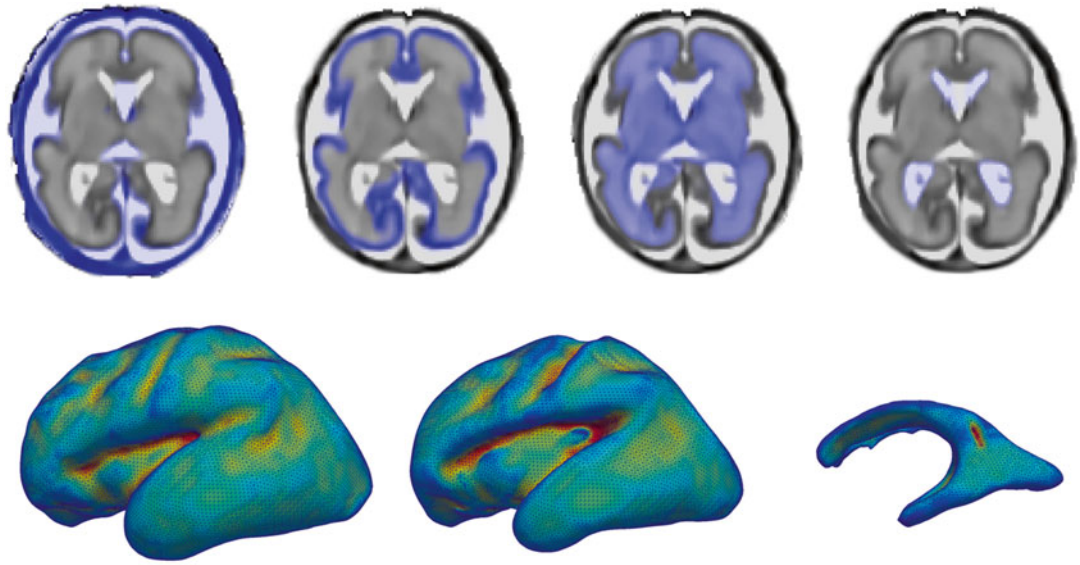

Fig. 20.2 *First row*: Segmentation result of a T2-weighted fetal brain MR image into four classes (CSF, cortical gray matter, white matter and deep gray matter, ventricles). *Second row*: surface meshes (brain surface, WM/GM interface, ventricles)

temporal model can be estimated locally (Dittrich et al., 2014; Habas et al., 2010)

Having the correspondence between each 3D point of each subject through the use of a reference anatomical template space enables a refined local analysis of the brain growth from MRI data. It is then possible to study quantitative parameters such as the change in curvature of the brain surface over time at any given location on the surface. Normative indices of in utero brain gyrification can be computed using an age-specific reference template (Clouchoux, Guizard, Evans, du Plessis, & Limperopoulos, 2012; Habas et al., 2012; Wright et al., 2014). More importantly, regions of the fetal brain surface that undergo significant folding changes during this developmental period can be identified. Habas et al. (2012) have provided precise temporal staging of these changes for each region of interest and have highlighted the emergence of inter-hemispheric structural asymmetries that may be related to future functional specialization of cortical areas.

Brain segmentation also enables us to study fetal brain growth at a regional level. In Scott et al. (2011), the volumes of major fetal zones (cortical plate, subplate and intermediate zone, germinal matrix, deep gray nuclei, and ventricles) were calculated from automatic segmentation of motion-corrected 3D reconstructed MRI. These new advanced image analysis techniques can help to build growth trajectories of the human fetal brain. One application of such work is the comparison of premature brain development and normal brain development in utero.

The formation of the complex structure of the cortex also can be studied from different growth patterns of brain regions. For this purpose, morphometric techniques based on the analysis of deformation fields can be used in association with a temporal linear model. In Rajagopalan et al. (2012), the authors made use of maps of the Jacobian determinant of the non-rigid transformations between anatomies at different gestational ages. These can locally quantify the differences in local anatomical size between the estimated average anatomy and the anatomy of each subject of the study. Local temporal linear modeling was used to detect brain regions for which the local tissue growth rate is higher (or lower) than the overall rate of brain growth (modeled by a global affine transformation). This approach, complementary to curvature analysis

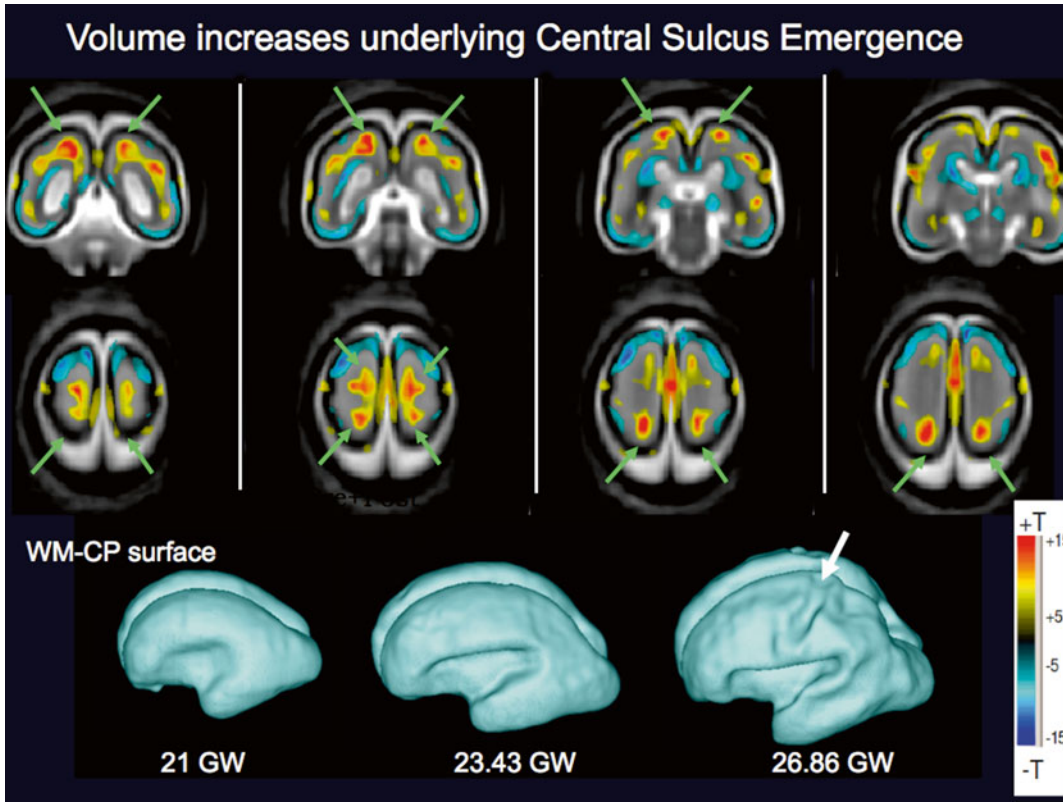

Fig. 20.3 Emergence of central sulcus. *Panel 1*: ortho slices demonstrating T maps ($P<0.05$) positive cluster localized to the central sulcus. *Panel 2*: 3D rendering of intermediate zone of three representative subjects

of the cortical surface, has particularly highlighted the process of accelerated regional tissue expansion underlying the process of gyrification of the cortical surface (see Fig. 20.3).

Diffusion Imaging

Diffusion MRI is an imaging technique that measures the diffusion of hydrogen nuclei over time. The protons of the water molecules contribute to the collected signal predominantly so it is mainly the diffusion of water molecules that can be observed by diffusion MRI. The local diffusion anisotropy is primarily due to axonal membranes, while myelin plays a secondary role in its modulation (for further details, see Chap. 8 by C. Beaulieu in Johansen-Berg & Behrens, 2013). Diffusion MRI allows visualizing microstructural properties of brain tissue. Since the pioneer work published in 1986 (Le Bihan et al., 1986), this imaging technique has been greatly improved in terms of MR sequence acquisition but also on data processing (Johansen-Berg & Behrens, 2013), and it is becoming a major tool for in vivo white matter studies, especially for early brain development study (Dubois et al., 2014). This imaging technique has been used for fetal brain imaging since the 2000s for the detection of cerebral ischemia as well as for the study of normal brain development (Prayer & Prayer, 2003; Righini et al., 2003).

Sequences

At each physical point in 3D space, thus, one can measure in a given direction a profile of the time-dependent diffusion. In order to maintain a short enough acquisition time in accordance with clinical constraints, one measurement of diffusion with a

fixed diffusion time is usually acquired. A typical study consists of the acquisition of a reference image with no sensitivity to diffusion (the so-called B0 image) and a set of diffusion-weighted (DW) images in non-collinear directions that allow the estimation of the local diffusion properties according to a specific diffusion model.

It has been shown that, under the assumption that the motion of water molecules is Gaussian and behaves according to the Einstein equation, there is an exponential relationship between the MR signal and the apparent diffusion coefficient (ADC; e.g., see Chap. 5 by D. K. Jones in Johansen-Berg & Behrens, 2013). ADC maps can be estimated based on only one DW image and the B0 image. A first attempt to study fetal brain maturation was published in 2003 (Righini et al., 2003) with the purpose to establish normal values of ADC in three regions of interest (frontal white matter, occipital white matter and basal ganglia). Fifteen fetuses were included in this study using a 1.5 T MRI unit. Because of frequent image motion degradation, two or three DW sequence acquisitions (with a resolution of $2.5 \times 2.5 \times 5$ mm^3 and a b factor set to 600 s/mm^2) had to be performed to obtain satisfactory image quality. It also has been shown that differences in diffusion anisotropy between gray matter and white matter could be visualized using diffusion MRI (Prayer & Prayer, 2003).

Since then, few studies have been published on the characterization of brain development using diffusion MRI (more specifically ADC measurements). They include the following works: Cannie et al. (2007) who measured ADC values in 46 fetuses (from 17 to 37 weeks of gestation) using 1.5 T MRI unit in basal ganglia, cerebellar hemisphere, frontal parenchyma, occipital parenchyma; Manganaro et al. (2007) who measured ADC in 56 fetuses (from 19 to 37 weeks of gestation) using 1.5 T MRI unit in basal ganglia, frontal and occipital white matter; J. F. Schneider et al. (2007) who studied ADC values in 78 fetuses (from 23 to 37 weeks of gestation) in the deep white matter of the centrum semi-ovale, the frontal, parietal, occipital, and temporal lobe, in the cerebellar hemisphere, the brainstem, the basal ganglia, and the thalamus; and finally, M.M. Schneider et al. (2009) who considered ADC values in 45 fetuses (from 21 to 33 weeks of gestation) in the periatrial white matter, frontal white matter, thalamus, basal ganglia, cerebellum, and pons. Regional differences in ADC values and their evolution over time reflect differences in brain maturation. However, although these studies provided (sort of) normative ADC values related to normal in utero brain development, it remains to understand the meaning of any deviations from this normal pattern and the functional impact later in life.

Diffusion MRI is an imaging technique that can provide diffusion measurements in any spatial direction. The ADC value depends on the direction in which it is measured. Therefore, a more complex model should be used in order to characterize diffusion. Under the Gaussian assumption, diffusion can be modeled using a tensor matrix, which is able to account for the spatial heterogeneity of the diffusion process. Such a diffusion tensor imaging (which requires data acquisitions in at least six directions) provides quantitative maps such as diffusion anisotropy (e.g., fractional anisotropy, FA), mean diffusivity, longitudinal diffusivity or transverse diffusivity. Diffusion tensor imaging has been performed on 15 postmortem fetuses with gestational ages of 15–37 weeks (Gupta et al., 2005). The study of diffusion anisotropy appeared then as a powerful tool in following neuronal migration or cortical maturation. Another study (Bui et al., 2006) has shown that diffusion MRI (and more specifically ADC and FA maps) could be done on in utero fetuses and that this imaging technique could provide quantitative assessment of the microstructural development of white matter.

More detailed studies taking into account all the information provided by the powerful and promising imaging technique remain difficult (Girard & Chaumoitre, 2012). The acquisition time of fetal brain DW images (with a resolution of $2 \times 2 \times 3.5$ mm^3) acquired in 30 directions in space, is nowadays about 7 min. All the studies previously cited do not make use of motion correction techniques. Acquisition time remains a limiting factor for clinical use of diffusion MRI for in utero fetal brain study.

Motion Correction

One of the flagship applications of diffusion MRI is the estimation of the white matter nerve fibers network (the so called connectome). The study of the connectome might provide a better understanding of the functioning and development of the brain but also certain diseases (neurodegenerative disorders, schizophrenia, autism, etc., Van Essen & Ugurbil, 2012). The quality of the connectome estimation depends heavily on data processing such as correction of distortions associated with diffusion acquisitions or subject motion correction. Subject motion generates a sparse distribution data acquisition, that is to say, scattering measurements obtained in different directions at different locations in 3D space. The goal is then to reconstruct diffusion images on a regular grid at fixed directions in order to apply classical diffusion models (such as rank-2 tensors; Minati & Węglarz, 2007).

Reconstruction algorithms dedicated to diffusion data are based on the same principle as the one developed for anatomical MR images. The distortion correction methods commonly used for adult imaging consist in registering the diffusion images with the B0 image (the latter serving as reference). Such a technique has been recently adapted to fetal diffusion data (Jiang et al., 2009) using a slice-to-volume registration method. However, since the B0 image can be very noisy, such approaches may not be robust enough for fetal imaging. By observing that the similarity between diffusion-weighted images is greater than the similarity between B0 image and any DW image, the average 3D diffusion weighted image was used as a reference image. In Oubel, Koob, Studholme, Dietemann, and Rousseau, (2012), each slice of DW images is iteratively registered to this reference volume by considering an affine transformation and mutual information as a criterion of similarity. Reconstructing data on a regular grid (in 3D space and the space of diffusion) is done with an interpolation method using two separable kernels (spatial and diffusion) where the angular distance is the geodesic distance on the unit sphere. The advantage of this approach lies in the fact that no constraints are imposed on the model of diffusion data and the reconstruction process is performed on diffusion-weighted.

To further improve the quality of the reconstructed motion corrected diffusion data, similarly to super resolution framework, an observation model can be used to model image formation in order to refine image details (Fogtmann et al., 2014). The algorithm incorporates image resolution modeling to iteratively deconvolve the effects of the imaging point spread function using the multiple views provided by thick slices acquired in different anatomical planes. The application of these reconstruction methods leads to detailed diffusion MR data allowing the study of fetal brain connectome. Figure 20.4 shows an example of a motion-corrected DW dataset where a local diffusion orientation map is superimposed with high-resolution motion-corrected anatomical image.

Tractography

Diffusion MRI is an imaging technique for visualizing the diffusion of water molecules. The motion of these molecules is anisotropic due to physical constraints induced by brain structures. The measurements of the diffusion of water provide, indirectly, information on brain structure and, more specifically, on the geometry of the white matter fibers. Diffusion MRI is a way to measure the diffusion propagator, that is to say the average propagation in a specific spatial direction. The most commonly used diffusion model (especially in clinical practice) is the diffusion tensor from which can be extracted easily interpreted information such as apparent diffusion coefficient (ADC) or fractional anisotropy (FA). However, it remains difficult to estimate complex fiber geometry such as fiber crossing only based on diffusion tensors. To overcome this issue, several diffusion models have been proposed: multi-tensor model, Q-ball model, high order tensor, etc. (e.g., see Chap. 6 by Seunarine & Alexander in Johansen-Berg & Behrens, 2013). These models can be used to recover fiber crossing when the angle between fibers is greater than 30°.

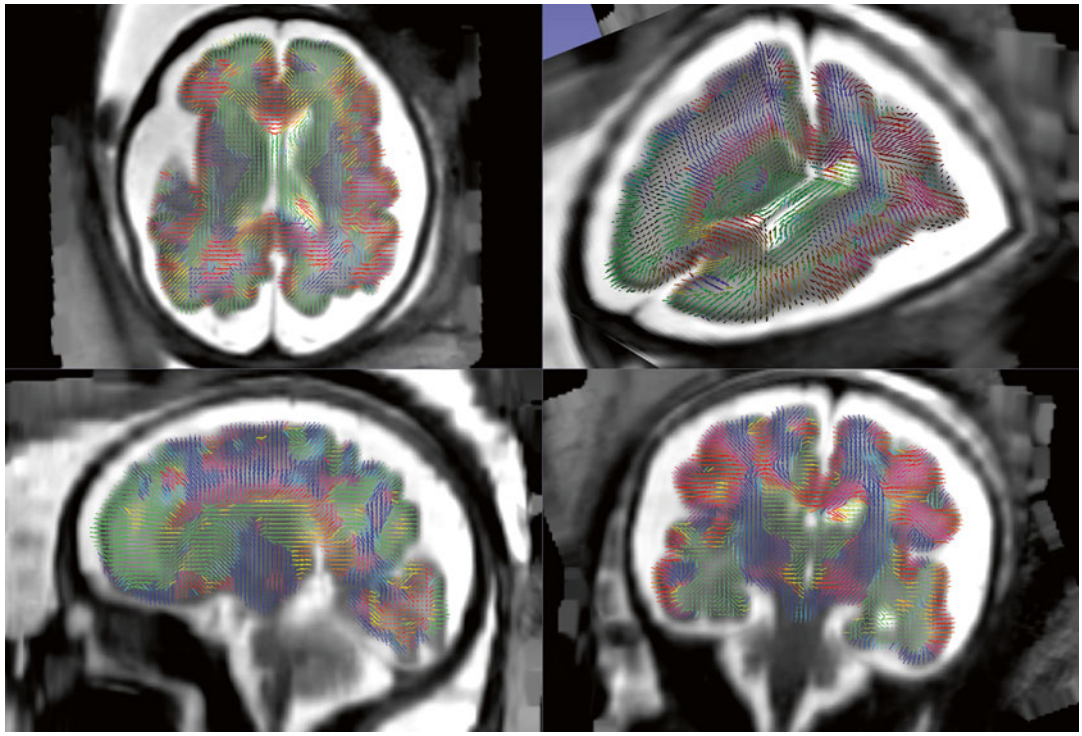

Fig. 20.4 Motion corrected diffusion MR fetal brain dataset (showing colored main local diffusion orientations) superimposed with high-resolution reconstructed anatomical image

Once diffusion model parameters are estimated at each voxel, tractography algorithms (see Lazar, 2010 and Chap. 19 by Behrens, Sotiropoulos & Jbabdi in Johansen-Berg & Behrens, 2013 for detailed reviews) can be applied to estimate main fiber bundles. The direction of the primary eigenvector of the diffusion tensor is believed to correspond to the main orientation of the underlying fiber bundles. Starting from a region of interest (ROI) defined by the user and connecting the primary eigenvectors leads to the reconstruction of the pathways of the white matter tracts. These ROI-based deterministic streamline propagation methods (Mori, Crain, Chacko, & Van Zijl, 1999) have been used to study the fetal brain connectome ex vivo (Huang & Vasung, 2014; Vasung et al., 2010) or in utero (Kasprian et al., 2008; Zanin et al., 2012). These studies have shown that diffusion MRI and tractography allow the visualization of the 3D appearance of the main commissural and projection tracts in the developing fetal brain in utero, in accordance with postmortem studies.

Although these studies have demonstrated the feasibility of tractography techniques applied on in vivo fetal diffusion MR data, the use of simple deterministic streamline techniques such as FACT (Mori et al., 1999) in conjunction with rank-2 tensor may be limited in providing accurate full fetal brain connectome estimation. As a result, a probabilistic approach using an analytical Q-ball diffusion model (Descoteaux, Deriche, Knösche, & Anwander, 2009) has been recently developed in order to provide more robust estimates of fetal brain connectome (Pontabry et al., 2013). The use of Q-ball model leads to more accurate estimates of fiber bundles and the probabilistic component of the algorithm provides uncertainty about the estimates. Tractography is mathematically expressed within a particle filtering framework by estimating the distribution of the system state (fiber parameters) at the current time knowing all the past and present observations (Zhang, Hancock, Goodlett, & Gerig, 2009). Figure 20.5 shows the fiber bundle network estimated from in utero motion-corrected diffusion MR data of one subject.

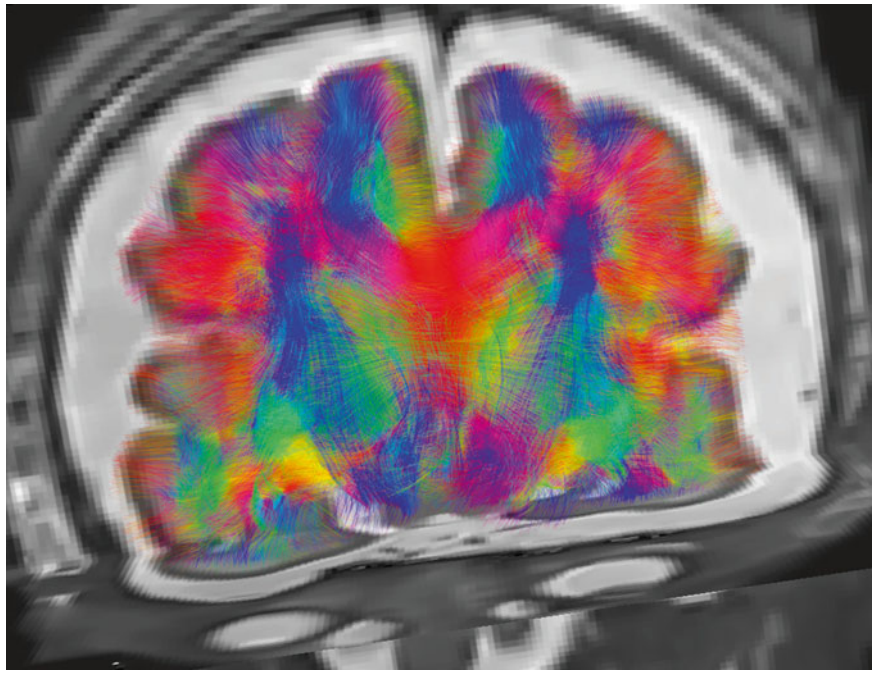

Fig. 20.5 Fiber tracts estimated from in utero motion-corrected diffusion MR data of fetal brain

Network Analysis

The developed brain diffusion data can be analyzed using graph theoretic methods to study the underlying structure of white matter connectivity. This has led to the discovery of small-world characteristics of structural brain connectivity (Gong et al., 2009; Iturria-Medina, Sotero, Canales-Rodriguez, Aleman-Gomez, & Melie-Garcia, 2008). These methods have more recently been applied to look at the growing brain and the development of structural small world connectivity (Fan et al., 2011) in healthy pediatric subjects at ages of 1 month, 1 year, and 2 years. These approaches are dependent on the ability to identify corresponding regions of the brain cortex and sub-cortex between which connectivity can be evaluated. In both adults and neonates, the presence of a consistent brain folding pattern with the same basic sulci present at all ages provides a basis for such studies. In mid-gestation, fetal development studied in typical clinical MRI scans, such consistent structural features are not yet present, as even the primary sulci are still undergoing formation. As a result, recent work by Cheng et al. (2013) has explored the use of alternative cortical partitioning schemes that probe the connectivity patterns using repeated random or regular partitioning to sample the connectivity across different scales of cortical organization. These have been shown to provide measures of developing network connectivity that support the observation of emerging small world structural network characteristics in the brain well before birth.

Functional Imaging

Functional MRI (fMRI) is a well-established tool for the exploration of neurological function and connectivity in adults and children (Bandettini, 2012). Recent technological developments have allowed fetal fMRI to emerge as a central method for investigating the development of human fetal functional brain systems (Anderson & Thomason, 2013). This section reviews both task-based and resting-state fMRI methods that have been used

to explore the highly plastic functional organization of the brain during prenatal life.

Sequences

T2* weighted MRI sequences based on *echo-planar imaging* (EPI) are able to capture changes in relative blood oxygenation that accompany neural activity. This so-called *blood-oxygen-level-dependent signal* (BOLD) has complex spatiotemporal properties driven mainly by nonlinear processes (Hillman, 2014). The characteristic pairing of increased neural activity and blood flow in the capillary bed is referred to as *neurovascular coupling*. While prior work demonstrates that local neuronal excitation and changes in fMRI signal are related (Lee et al., 2010), the physiological basis of neurovascular coupling in the human fetus is not well understood. Despite this limitation, seminal prenatal fMRI studies have identified key components of the hemodynamic response in the fetal brain exposed to sensory stimulation, using respectively 0.5 (Moore et al., 2001) and 1.5 T MR field strengths (Jardri et al., 2008). In particular, the rise and peak-plateau of the hemodynamic response were evident in utero (Jardri et al., 2008; see Fig. 20.6). The observation that fetal hemodynamic response mirrored what had been observed postnatally had tremendous value; it provides assurance that fMRI can be used to indirectly measure neural function in the developing human fetus.

Despite gross similarities in the hemodynamic response function, kinetics of fetal BOLD signal may differ somewhat from those observed in children and adults. This is not all too surprising given the interdependence of fetal and maternal circulations, immature physiological scaffolding, and high proportions of Hemoglobin ε and F in the human fetus. A recent relaxometry study focused on key anatomical regions of the fetal brain and evidenced larger T2* values than previously reported in neonates as well as a trend to decline across gestational age (Vasylechko et al., 2014). These findings suggest that optimal BOLD sensitivity in fetal fMRI may require adjustments, including longer than usual echo times or direct T2* measurement.

Advances in functional MRI may be transformative in our ability to access information about fetal brain development in utero. For example, it has been noted that standard fMRI spatial resolution may be inadequate for the fetal brain, which is small in size. In the last decade, EPI variants have been developed to overcome the limitations of spatial resolution while maintaining sufficient signal to noise ratio (SNR). fMRI spatial resolution can be improved by either reducing the imaging *field-of-view* or by increasing the image acquisition rate. An example of reduced field-of-view EPI is *zoomed-EPI*

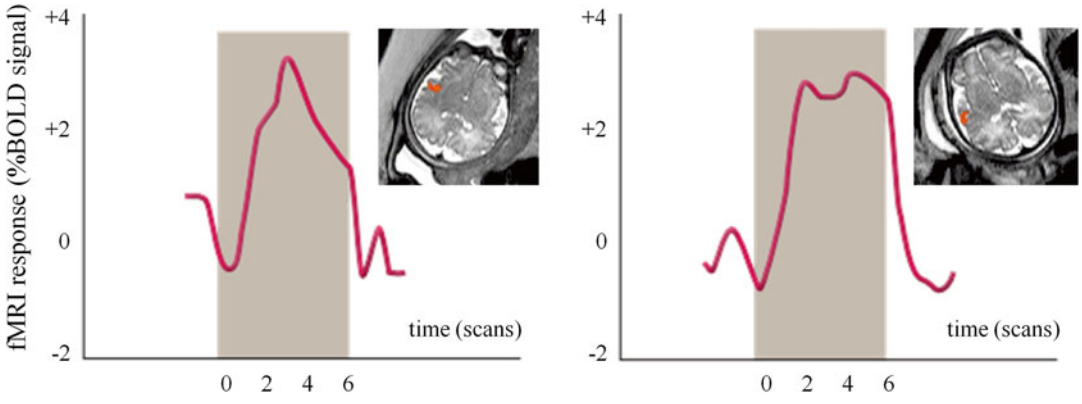

Fig. 20.6 Hemodynamic response computed across the left temporal lobe for two fetuses exposed to auditory stimulation at 33 weeks of gestation (adapted from Jardri et al., 2008). The average length of time is expressed as a percentage of the BOLD signal per scan (each scan lasted for 3 s). The *brown bar* represents the stimulation period

(Pfeuffer et al., 2002) that allows restriction of the signal volume in order to increase resolution without increasing the number of phase encoded echoes, without signal aliasing (Feinberg & Yacoub, 2012). An alternative to this is multi-band EPI that enables accelerated simultaneous excitation and slice acquisition, greatly decreasing imaging times (Xu et al., 2013). These advances, and others (e.g., multi-echo EPI, Kundu, Inati, Evans, Luh, & Bandettini, 2012), in BOLD EPI may usher in a new era of human fetal functional imaging, but this remains to be determined, as these methods have scarcely yet been deployed in a developmental sample, let alone in a fetal sample.

Fetal fMRI Preprocessing Challenges

The conventional preprocessing pipeline required for fMRI analysis needs to be adapted to the fetal context, since specific sources of noise may effectively lead to a high rejection rate of volumes within the acquired data-set (40 % on average, Hykin et al., 1999; Jardri et al., 2008; Jakab et al., 2014; Moore et al., 2001; Schöpf, Kasprian, Brugger, & Prayer, 2012). As an example, *geometrical distortions* caused by magnetic field inhomogeneity are less pronounced in utero than after birth since the fetal head is surrounded by amniotic fluid or maternal soft tissues and not air. In contrast, *motion correction* appears to be the main challenging problem due to the sporadic and unpredictable nature of fetal head motions but also indirectly to maternal respiration. When the fetal head moves, neurons under a recorded voxel move and hence their time-course largely represents that of some other voxels in the past. Up to now, fetal fMRI has been limited to analyzing dynamics during periods in which motion is minimal.

Two types of motion correction have been developed to correct trace amounts of movement and inaccuracies due to shifts in timing. The first strategy, called *rigid-body transform*, acts at the level of the whole fMRI volume to account for motion. The *rigid-body transform* shifts and/or rotates the processed volume and compare it with the reference volume[1] using a cost function. Readers may note that a second conventional preprocess called *Slice-Timing Correction*, supposed bringing all the slices acquired during a brain volume to the same timepoint, is not recommended in the fetus, since it may propagate distortions and artifacts related to the disturbed magnetization history of the voxels to the adjacent slices (Jardri et al., 2008). Because 3D rigid-body correction may fail in case of massive translation/rotation, some authors completed this step looking through the brain volumes like a film and discarding volumes in which persistent motion was registered (i.e., <1.5° or >2 neighboring pixels in *x*/*y*/*z*; Jardri et al., 2008; Moore et al., 2001). If the removal is performed on the raw data, the volumes kept can be automatically corrected using the *rigid-body transform*. The efficacy of this two-step procedure can be checked again on the brain-volume movie.

A second possible strategy is to correct motion on a slice-by-slice timescale (i.e., within fMRI volumes). This framework considers that the assumption made in 3D rigid-body corrections, of a negligible intra-stack movement, is frequently violated, which again may lead to a high rejection rate of data. Along this line, Ferrazzi et al. (2014) proposed a combined approach of slice to volume registration and scattered data interpolation with bias field and spin history corrections. Even if still preliminary, this method also has the advantage to place all the data into a consistent anatomical space. Seshamani, Cheng, Fogtmann, Thomason, and Studholme (2014) have also developed a method for intensity inhomogeneity removal in fMRI studies of a moving subject. Subtle changes in signal as the subject moves in the presence of a bias field can be a significant confound for BOLD signal analysis. In this work, a parametric bias field model is assumed and a regression model is used to estimate the basis function weights of this model.

[1] Reference volume can be the first time-point, but when T2 morphological recordings are acquired before and after the EPI sequence, the reference volume can be the closest axial T2 scan.

Hypothesis-Driven Versus Data-Driven Analysis Methods

The first fMRI studies were performed near to term (Hykin et al., 1999; Moore et al., 2001) and referred to a standard voxel-wise *hypothesis-driven* method for analysis of fMRI data-sets. Hypothesis driven analyses (HDA) evaluates data with reference to a hypothetical model for presentation of the stimulus. Although this has proven to be an effective approach, there are nevertheless a number of limitations when using it in fetal imaging. Firstly, since HDA is a univariate regression method, the problem of multiple comparisons of the data should be taken into account, by correcting the statistical thresholds, in order to avoid a large number of false positives (Genovese, Lazar, & Nichols, 2002). These corrections may cause the activation cluster-size to be reduced, or the smallest activations to be deleted. Secondly, HDA is sensitive to motion artifacts, which again, constitutes one of the major problem in fetal imaging (Gowland & Fulford, 2004). Finally, HDA is particularly sensitive to unpredictable events, for example a decrease in the performance of the participants in the study during testing (Quigley et al., 2002), which are common during fetal life, due to rapid waking–sleeping cycles.

Data driven fMRI analyses approaches, such as *Independent Component Analyses* (ICA), appear suitable compliments for studying fetal cerebral activity. ICA allows the multiple signal sources in the raw data to be separated out blindly, and is flexible enough to detect temporal structure that is difficult to model a priori (McKeown, Hansen, & Sejnowski, 2003). Indeed, ICA was superior to HDA in identifying fetal cortical activity during auditory stimulation (Jardri et al., 2008). In this work it was not necessary to predefine regions of interest or specify which were the stimulation and resting periods for each fetus before removing volumes to correct for motion (Jardri et al., 2008). Furthermore, automatic classification algorithms have been proposed to identify the specific components that capture neurophysiological related processes (e.g., De Martino et al., 2007), making separation of noise from signals of interest ever more accessible.

Task-Evoked Fetal Brain Activations

Task-based fMRI experiments were essentially proposed to explore early sensory processing and overcome the potential biases of fetal behavioral measurements, subject to many endogenous and exogenous influences (Fulford et al., 2003, 2004; Hykin et al., 1999; Jardri et al., 2008; Moore et al., 2001). For acoustic stimulation, the device is usually made from one of the earpieces of MRI-compatible headphones, fixed on the maternal abdomen using an elastic belt and surrounded by an MRI body coil (see Fig. 20.7a).

Importantly, the device used needs to face the fetus' head, which can be located by palpation or from the data of a follow-up ultrasound scan of the pregnancy (Jardri et al., 2012). The sound level coming out of the headphone needs to be calibrated to cover the noise of the scanner during the EPI sequence and assure that the fetus would be able to detect the stimulus without risking cochlear damage. Thanks to the attenuation of the sound by the successive maternal and fetal tissue barriers, 100 dB SPL is usually judged optimal (Jardri et al., 2008). For light stimulation, a red LED cluster transmitted through a cardboard tube lined with non-conducting aluminized plastic has been used, allowing for an intensity of 1100–1200 lx at the maternal abdomen (Fulford et al., 2003).

Initial studies referred to block designs. fMRI reports based on HDA, first allowed a measure of the brain response to light (Fulford et al., 2003) or sounds (Hykin et al., 1999; Moore et al., 2001) in fetuses near to term. Even if successful, these seminal studies had poor localization power, mainly due to the low magnetic field strength used (i.e., 0.5 T). As an example, the fMRI study that explored visual processing in 36–40 week gestation human fetuses failed to localize responses in V1 to visual stimuli (Fulford et al., 2003). Following experiments, conducted earlier during the third trimester, were able to answer two complementarily questions: (1) is there a cortical response distinguishable from reflex at 32 weeks of gestation (Jardri et al., 2008); and (2) is the fetus capable of specific sensory processing such as familiarity processing (Jardri et al., 2012)?

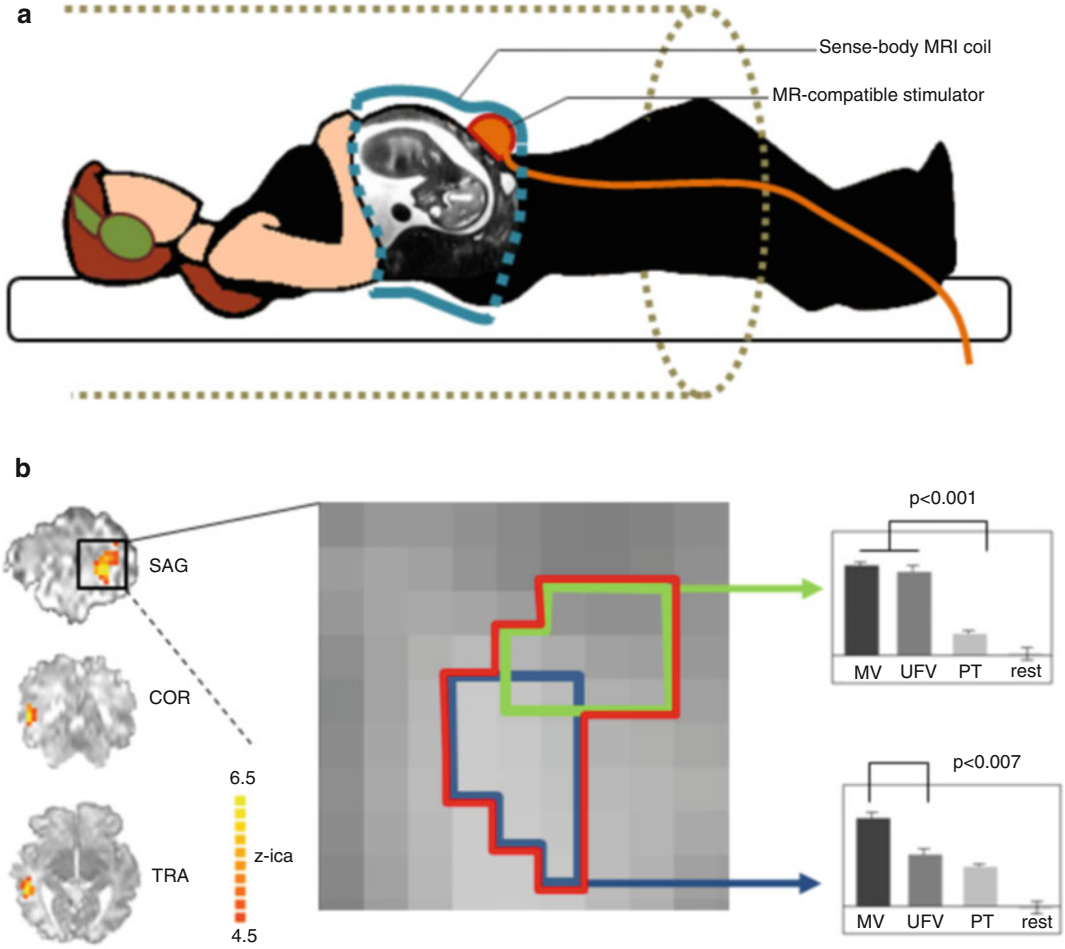

Fig. 20.7 Fetal brain responses to auditory stimuli (From Jardri et al., 2012). (a) Experimental setting. (b) Activation measured in the left superior temporal lobe in a 34-week gestational age fetus during auditory stimulation (*left panel*). The *green cluster* represents an increased response to speech compared with tones, while the *blue cluster* denotes a region displaying significant increases in activation during exposure to the maternal voice (*middle* and *right panels*)

This last question directly referred to the seminal work by Decasper and Fifer who showed in the 1980s that newborns, quickly after birth, preferred their mother's voice to other unfamiliar voices (DeCasper & Fifer, 1980).

Different auditory conditions were used in these experiments: pure tones and rest in the first one, and pure tones with two speech stimuli (one unfamiliar, the other one recorded from the mother) in the second experiment. For three of the six 33-week fetuses scanned, left temporal activations were evidenced for the pure tone condition (see Fig. 20.6, Jardri et al., 2008). In a subsequent sample, using the same procedure but with different stimuli in six pregnant women, left fetal temporal activations for pure tones at 33 weeks of gestation were confirmed (see Fig. 20.7b). By further exploring the activation pattern induced by the mother's voice, this report also revealed a selective fetal cortical processing for the maternal voice at 34 weeks GA (Jardri et al., 2012), consistent with previous observations of fetal heart-rate responses to the maternal voice between 32 and 34 weeks GA (Kisilevsky & Hains, 2011). Specifically, activation in the upper bank region of the left temporal lobe was observed in

response to unfamiliar voices while lower bank regions selectively responded to the maternal voice in a 34-week gestational age fetus. Even if very preliminary, this finding appears compatible with the possible existence of in utero associative learning.

Intrinsic Functional Connectivity of the Fetal Brain

A second set of experiments referred to the spatiotemporal distribution of fetal neural networks at rest. In adults, these Resting-State Networks (RSN), spontaneously oscillate below 0.1 Hz and were shown separable on the basis of independent temporal characteristics using ICA or correlation between a seed and other voxels in the brain (Biswal, Yetkin, Haughton, & Hyde, 1995; Cordes et al., 2001). Damoiseaux et al. (2006) found auditory and visual RSNs in the human brain, but one RSN is characterized by its unique response to cognitive tasks (i.e., the "default-mode network," DMN, Fox et al., 2005), including the bilateral inferior parietal lobules and anterior/posterior cingulate cortices. The DMN also was identified in preterm neonates at a mean gestational week of 41 (Fransson et al., 2007), and appeared to follow dynamical changes in the connection strength between its constituting nodes during postnatal development (Fair et al., 2008). Exploring RSNs in utero may provide a unique way of investigating the maturation of functional networks in task-free fetal states.

Using single-subject ICA to analyze resting-state fMRI data acquired in 16 fetuses from the gestational weeks 20–36, Schöpf et al. (2012) evidenced bilateral occipital and frontal networks oscillating within the 0.01–0.06 Hz range. Based on group ICA and seed-based connectivity analyses, Thomason et al. (2013) further explored age-related changes in precursors of RSNs, such as motor or modality-dependent sensory networks, in 25 healthy human fetuses in the second and third trimesters of pregnancy. This experiment provided evidence that more than 80 % of the areas examined demonstrated increased cross-hemispheric connectivity with advancing gestational age, mirroring increased connectivity between the right and left hemispheres that occurs in this time frame. Higher fMRI connectivity also was evidenced in medial brain regions (see Fig. 20.8), supporting a medial to lateral connectivity gradient in the fetal brain.

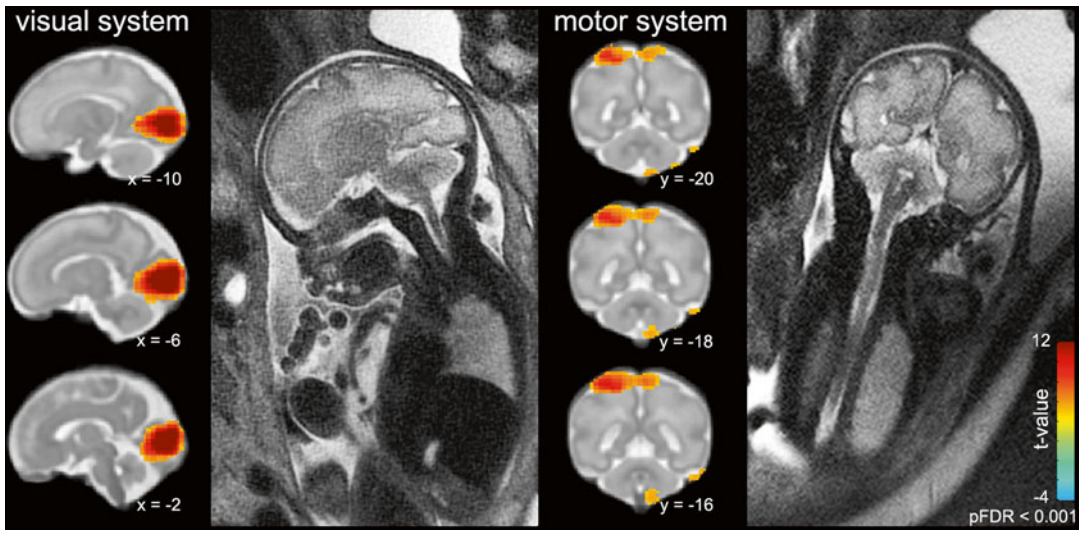

Fig. 20.8 Demonstration of fMRI connectivity of visual and motor neural systems in the human fetal brain in utero. One sample *t*-tests performed in 32 fetuses between 25 and 37 weeks of gestation age were used to extract group connectivity averages using a $P<0.001$, false discovery rate corrected, statistical threshold. Results are displayed on a 32-week fetal template for anatomical reference, and presented adjacent to a healthy 32-week fetus studied with fMRI in utero. Robust connectivity across primary and secondary visual regions and cross-hemispheric motor and cerebellar functional connectivity were observed

Exploring RSNs using *Graph Theoretical Analyses* in 33 human fetuses between 19 and 39 weeks of gestation, the same team confirmed that the fetal brain modularity decreases while the connectivity of the posterior cingulate cortex (considered as a rich-club constituting node of the DMN) becomes more negative with advancing gestational age (Thomason et al., 2014). In their most recent work, Thomason and colleagues (2014) describe the emergence of several proto-RSNs in 39 fetuses and provide empirical evidence that long-range, cerebral–cerebellar, and cortical–subcortical connections begin to form in utero. By mimicking functional principles observed postnatally, these findings support the early emerging capacity for information processing, as evidenced in task-based experiments.

Conclusion

This chapter covers the main recent advances in MRI enabling us to study the human fetal brain in utero. Since its beginnings, MRI was found to be an effective imaging modality to visualize fetal brain anatomy in detail. However, despite significant improvements in MR sequence design, including acquisition time reduction, the study of fetal brain from MRI remained difficult. We have shown in this chapter that thanks to new techniques from image processing developed during the last few years, it is now possible to correct fetal motion and thus reconstruct MRI data in 3D. Several examples of structural MRI studies, but also in diffusion MRI and functional MRI, have been presented, fully demonstrating the potential of MRI for early brain development study. Motion correction methods open a route for expanding the use of MRI in fetal brain studies, in particular to enrich the information from images by considering other MRI techniques such as magnetization transfer or relaxometry. Further work will be interdisciplinary studies linking MR image based findings to microscopic studies of cell growth (Lui, Hansen, & Kriegstein, 2011), biomechanical models of brain growth (Taber, 2014), or neurodevelopmental outcomes (Larroque et al., 2008).

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Functional Imaging of the Prenatal Brain

21

Veronika Schöpf, Georg Langs, and Andras Jakab

Abstract

In utero magnetic resonance imaging (MRI) has significantly increased our knowledge on early fetal brain development. Especially the possibility to expand standard clinical applications of imaging structure to functional imaging has increased the opportunities but also introduced major challenges in the field regarding motion artifacts, group analysis, and generating structural templates. This chapter gives an overview on fetal functional imaging from stimulation to resting-state studies and discusses critical challenges in data analysis. Fetal functional MRI is a powerful approach investigating brain development in utero and has the potential of generating biomarkers for developmental prognosis in the future.

Fetal MRI

Magnetic resonance imaging (MRI) in living human fetuses has revolutionized the ability to investigate human brain development in vivo. Initial measurements in the early 1980s (Smith, Adam, & Phillips, 1983) cleared the way for the application of this noninvasive measurement technique, which has continuously improved ever since (Brugger, Stuhr, Lindner, & Prayer, 2006). While the use of sedative medication was long deemed indispensable for diagnostic fetal MRI, the development of ultrafast MR sequences (Chen & Levine, 2001; Lan et al., 2000; Yamashita et al., 1997) led to acquisition times of less than 20 s and reduced the problem of fetal motion artifacts for diagnostic sequences. A complete MR examination of a living human fetus today can be performed within 45 min, without sedating the mother or the fetus (Brugger et al., 2006).

According to follow-up studies of infants or children who had been exposed to MR imaging in utero, no gross abnormality, disease, or disability likely to be related to MR exposure could be demonstrated at the age of 9 months

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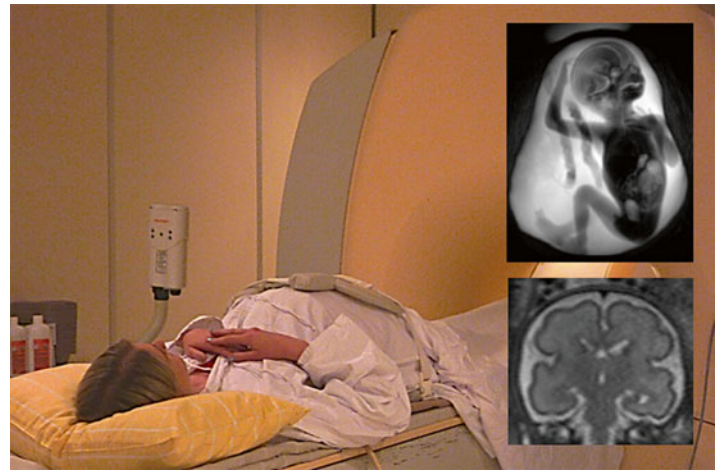

Fig. 21.1 Fetal MR acquisition. The *large picture* depicts a woman prepared to undergo a fetal MR acquisition in the supine position using a cardiac coil. *Upper right*: Survey of the fetus in utero; *lower right*: T2 coronal slice of a fetal brain in utero

(Brewerton, Chari, Liang, & Bhargava, 2005), 3 years (Kok, de Vries, Heerschap, & van den Berg, 2004; Smith et al., 1983), or 8 years (Kok et al., 2004). Even MR exposure at early embryonic stages was not associated with a higher risk for cytotoxic, teratogenic, or anti-proliferative damage (Kanal, Gillen, Evans, Savitz, & Shellock, 1993; Schwartz & Crooks, 1982; Supino et al., 2001). As the MR-related noise is grossly absorbed by the amniotic fluid (Glover et al., 1995), no abnormalities in the hearing function of infants who had undergone MRI in utero have been observed (Kok et al., 2004). For pregnant women undergoing fetal MRI stress coping strategies and whether women are accompanied or not play an important role in the experience of anxiety and depressive symptoms (Derntl et al., 2015). These factors should be considered especially in patients with high-risk-pregnancies to improve patient care. The Safety Committee of the Society of Magnetic Resonance Imaging issued the following guidelines and recommendations for MRI during pregnancy (Shellock & Kanal, 1991): "MRI is indicated for use in pregnant women if other non-ionizing diagnostic imaging methods are inadequate or if the examination provides important information that would otherwise require exposure to ionizing radiation. It is required that pregnant patients be informed that, to date, there has been no indication that the use of clinical MR imaging during pregnancy produces deleterious effects." According to the literature, in cases where the referring physician and attending radiologist can support the fact that the findings of MR imaging would have the potential to affect the care of the mother or the fetus, fetal MR imaging may be performed with oral and written, informed consent, regardless of the trimester (Shellock & Crues, 2004).

The availability of advanced imaging techniques, such as functional magnetic resonance imaging (fMRI) and tractography using diffusion tensor imaging (DTI) have paved the way from pure anatomical and morphometric imaging to a more integrated understanding of fetal brain development (Fig. 21.1) (Mailath-Pokorny et al., 2012; Schöpf et al., 2013).

Functional Magnetic Resonance Imaging (fMRI)

Functional magnetic resonance imaging is the most important noninvasive method with which to investigate brain function in the living human. In a clinical routine setting, it is commonly used for the localization of specific functional cortical areas in pre-surgical planning for brain tumor resection or epilepsy treatment. Moreover, fMRI also offers information about the spatial distribution and interaction of neural processing networks, characterized by spontaneous low-frequency fluctuations (<0.1 Hz). These so-called

resting-state networks (RSNs) have revolutionized neuroscience research by offering a model of the "default" functional connectivity of the brain (Biswal, 2012; Lowe, 2012; Raichle & Snyder, 2007; Snyder & Raichle, 2012). As such, they are thought to form the basis for the processing of versatile inputs such as sensory, cognitive, emotional, and motor functions (Rosazza, Minati, Ghielmetti, Mandelli, & Bruzzone, 2012). RSNs are observed throughout the whole brain and have been demonstrated to hold specific spatial and temporal characteristics independent of patient condition (sleep, task performance, rest, anesthesia, age). Most importantly, they also are hypothesized to serve as an imaging modality in neurodegenerative studies correlating RSN characteristics with disease progression. From pure fundamental research and proof-of-principle studies, this method has created a whole new tool for practical clinical application, especially for pre-surgery planning (e.g., epilepsy), as it does not require active interaction with the patient/subject.

Driven by the need for a better prognostic evaluation of fetal brain pathologies, fMRI has recently entered the field of prenatal imaging.

Fetal fMRI

Within a decade of the discovery of blood oxygen level dependent (BOLD) fMRI (Ogawa, Lee, Kay, & Tank, 1990) in 1990, and the first demonstration of detecting brain activity with BOLD (Ogawa, Tank, & Menon, 1992), the first fetal functional MRI experiment was conducted in 1999 by Hykin et al. As in utero conditions do not allow for cooperation of the subject, stimulation designs are very limited. Some studies utilized conventional fMRI paradigm design and stimulation by performing visual (Fulford et al., 2003), auditory (Hykin et al., 1999; Jardri et al., 2008, 2012; Moore et al., 2001), or vibroacoustic (Fulford et al., 2004) tasks. Results from those studies were highly variable and led to the conclusion that conventional analysis methods and standard stimulation designs might not be expedient for investigating fetal brain function. This relates to the fact that an appropriate paradigm design, in terms of block length and stimulation sequence, is largely unexplored and that reported studies applied their stimulation design based on the knowledge of sensory systems in adults. Furthermore, the hemodynamic response function (HRF), which is defined as the prediction function of the brain response to a certain stimulus, is supposed to be substantially unpredictably different in the fetal brain. Therefore, standard analysis tools dealing with a predefined HRF, adequate for the analysis of ex utero fMRI data, might lead to false-positive results.

A possible way to overcome the question of stimulation designs and to investigate the developing human connectome, is resting-state functional imaging. Resting-state fMRI studies have successfully shown, on a single subject basis, that RSN patterns are formed in utero (Schöpf, Kasprian, Brugger, & Prayer, 2012; Schöpf, Kasprian, Schwindt, Kollndorfer, & Prayer, 2012), as well as on a group level (Thomason et al., 2013).

Furthermore, a combination of functional and structural information about the developing brain may influence the detection of maturational changes and provide deeper insight into the temporal trajectory of brain development. Information about these maturation processes could aid in the accurate prognosis of how these changes may shape sensory, motor, and cognitive abilities. Although fMRI has provided a wealth of new information in adults, it has less frequently been applied to the developing brain, as the data analysis is very challenging.

Data Analysis

The low signal-to-noise ratio of BOLD functional MRI necessitates special attention and versatile image processing strategies to mitigate the effects of nonneural confounding sources. For the analysis of fMRI-based functional neural connectivity (Biswal, 2012), we rely on the lowest frequency bands of the observed signal variation (e.g., 0.01–0.1 Hz). Hence, noise sources within this frequency range may induce spurious

correlations of the BOLD time series and lead to incorrect conclusions regarding regional functional connectivity. Although our knowledge of the hemodynamic properties of the fetal brain is limited, it is safe to assume that similar confounding sources contribute to false-positive observations in fMRI analysis, such as physiological oscillations due to respiration and cardiac cycle (Krüger & Glover, 2001). Furthermore, in-scanner movements of the participant lead to nonstationary noise processes by two phenomena: displacement of image voxels; and, spin history artifacts (Friston, Williams, Howard, Frackowiak, & Turner, 1996). The first category of movement-induced signal confound is the function of brain voxel positions in the reference space of the scanner, and can most feasibly be corrected by realigning image frames to a reference set within the acquisition (Jenkinson, Bannister, Brady, & Smith, 2002). Spin history artifacts depend on the history of excitation experienced by magnetic spins within the voxel and consequent changes in local saturation (Friston et al., 1996).

Current fetal fMRI acquisition strategies do not require the sedation of the mother or the fetus. Therefore, fetal or maternal movements are challenging to control retrospectively. Spontaneous movements of the limbs, trunk, or head follow the neural maturation of the fetus and are dependent on fetal state of arousal (De Vries, Visser, & Prechtl, 1982), introducing a major problem and are often a criterion for exclusion of imaging data during the second and third trimesters of gestation. As of 2014, a significant proportion—approximately 50–75 %—of time-resolved fetal MRI data (e.g., fMRI or diffusion tensor imaging) have to be excluded from further analysis because of fetal motion-related errors.

The typical analysis work-flow to retrospectively correct head motion in fMRI data is to (1) differentiate non-brain tissue on the images using specialized image segmentation tools for prenatal anatomy, (2) estimate the frame-by-frame displacement of the fetal head by consecutive image registrations, and (3) use the obtained motion estimates to correct for signal changes induced by the spin history artifacts. Prior to connectivity analysis, censoring data to exclude periods of high motion is practical by an approach called *scrubbing* or *de-spiking*. Fetal fMRI is based on single-shot multi-slice EPI scans in which 2D slices are acquired at sequential intervals. Therefore, subject motion can be present between individual slice acquisitions as well, and global brain image realignment is not sufficient for correcting fetal head motion artifacts (Fig. 21.2).

To minimize the confounding effect of head motion, the following solutions are offered for fetal fMRI or other time-resolved MRI. These can be realized by either adapting neuroimaging pre-processing tools to the fetal age (such as the SpaceTimeRealigner algorithm in the Nipype package (Gorgolewski et al., 2011)), or by the use of MCFLIRT in FSL (Jenkinson et al., 2002), or by applying specialized toolboxes for fetal image analysis (BTK: Rousseau et al., 2013). Fetal fMRI can be processed through four-dimensional registration algorithms, by simultaneously correcting for motion and slice timing (Roche, 2011). Slice intersection motion correction is an approach that seeks to directly co-align multiple slice stacks by considering the matching structure along all intersecting slice pairs in all orthogonally planned slices that are acquired (Kim et al., 2010). A more complex processing pipeline includes distortion correction, frame motion estimation, and two levels of slice-to-volume registration, implemented as cascaded slice-to-volume registration (Seshamani et al., 2013).

Fetal fMRI data is presumably altered by nonneural and non-motion-related signal confounds, which are regarded as physiological noise. Algorithms to removing cardiac and maternal respiratory-related noise include temporal filtering, image based retrospective correction (RETROICOR), or navigator echo-based correction, albeit there is no literature regarding the optimal nuisance removal approach for fetal data. As an example, anatomical priors can be used to record signal, where we assume no neural signal, and the respective time courses of such image parts are regarded as nonstationary noise. Promising approaches for nuisance correction are CompCor (Behzadi, Restom, Liau, &

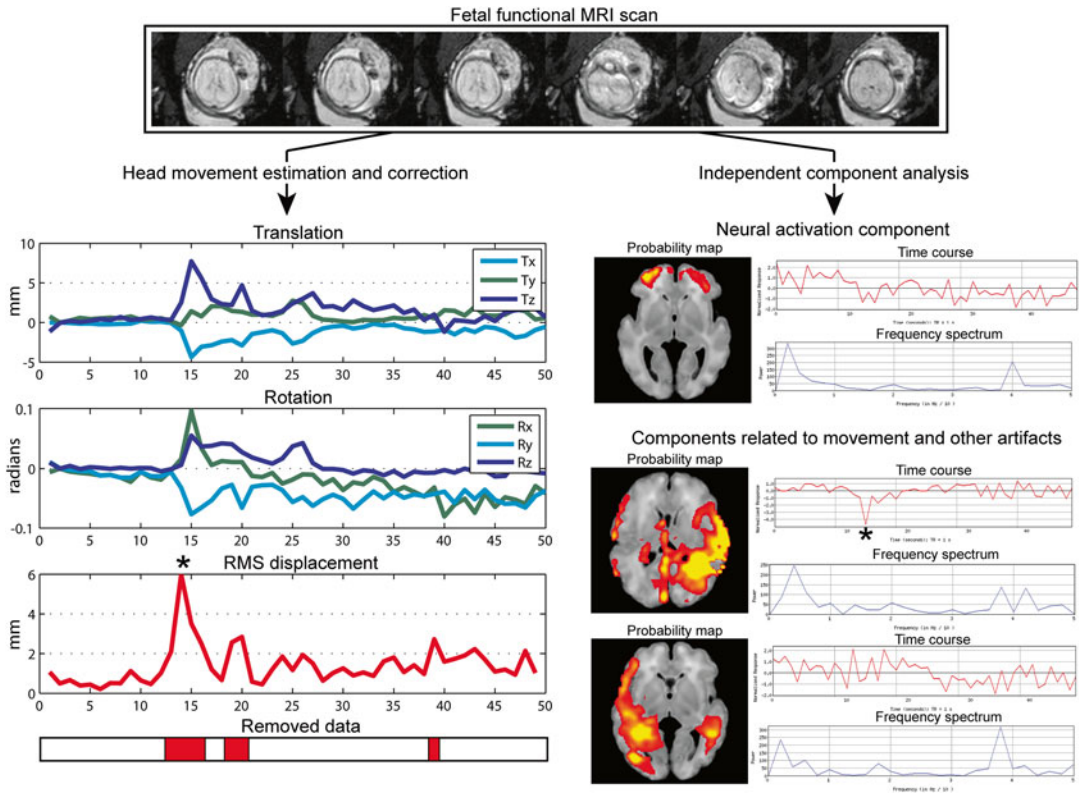

Fig. 21.2 Fetal functional MRI. Motion-related errors and artifacts in imaging. Fetal fMRI is a challenging technique, which is hindered by intra-scanner movements and hardware related artifacts. In this figure, an example is given for such artifacts. *Top image*: example frames from the fMRI of a 34-week-old fetus. *Left panels*: head motion is estimated using image registration algorithms, such data are used to remove periods of excessive fetal movement. *Right panels*: independent component analysis separates spatially coherent functional networks from fMRI data. This technique is used to visualize and identify presumably neural signal sources ("neural activation component") and artifacts as well. As illustrated, large fetal head movement induces spurious changes in MRI signal intensity, which manifests in false positive findings (asterisk: large movement of fetal head and the corresponding signal change in the fMRI scan)

Liu, 2007), in which algorithm noise components are estimated using principal component analysis, or FIX (Salimi-Khorshidi et al., 2014), where spatially coherent noise components are discovered and reduced with independent component analysis.

Fetal fMRI holds promise as a quantitative, prenatal imaging biomarker for diseases that disrupt normal neural functioning. For such purposes, it is necessary to achieve anatomical correspondence across study subjects. In postnatal MRI studies, this is most commonly done by aligning brain images to anatomical templates incorporated into atlases. Registering the fetal brain to anatomical templates (Dittrich et al., 2014) requires an algorithm that can tackle the problems of rapid morphological changes during development and encourage the construction of fetal developmental atlases.

Group Analysis

In many neuroimaging studies, establishing correspondence across individual observations is a prerequisite for group analysis. In adults, reference spaces, such as those introduced by Talairach and Tournoux (1988), or the Montreal

Neurological Institute (Evans et al., 1993), serve as means to match spatial positions in multiple subjects or patients, using software such as FreeSurfer (Fischl et al., 2002), or FSL (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). Once matching is achieved, local measurements can be compared across individuals, or region labels can be transferred from a label template or atlas to individual cases. Labels can be transferred either by mapping them directly, or by using a probabilistic atlas as a prior when segmenting imaging data. In addition to using an atlas as a means to process new data, the atlas itself is informative regarding the population from which it is constructed.

While atlases are widely used in adult neuroimaging studies, they are relatively new in fetal neuroimaging. This is, in part, due to the challenge of creating a representative template and atlas from a population that varies not only due to natural cross-sectional differences but also due to substantial developmental changes of shape and appearance (Serag et al., 2010) over a short period of time (Dittrich, Kasprian, Prayer, & Langs, 2011). In Kuklisova-Murgasova et al. (2011), a probabilistic atlas for gray and white matter, corticospinal fluid, and brain stem was built from 142 neonatal subjects with gestational ages from 29 to 44 weeks. For each gestational age, the reference space and corresponding labeling was obtained by kernel regression in the atlas population along the age axis. In a similar approach, Habas et al. (2010) created a spatiotemporal probabilistic atlas of fetal brain development for a period from gestational week (GW) 20 to GW24. They performed group-wise registration of structure annotations across individuals and modeled the nonlinear change of appearance and shape across the gestational period, and showed that the resulting model improved segmentation of new cases, when used as a prior in addition to image based segmentation.

Annotations of reference cases might be unavailable, or difficult to obtain, due to variability in the imaging appearance of brain structures across individuals, the limited contrast between different tissue types, or the necessity for a large number of examples due to the high variability of the anatomical structure of interest. Here, the computational analysis of the atlas population itself becomes a central part of atlas building. The so-called latent atlases (Riklin-Raviv, Van Leemput, Menze, Wells, & Golland, 2010) collect information from observations made across different examples, and learn a prior for the segmentation of specific structures in individual cases. In Dittrich et al. (2014), the authors extended this approach to spatio-temporal latent atlases, and used it to learn segmentations of the brain structure in fetuses from GW20 to GW30. The resulting spatio-temporal latent atlas allows not only an estimation of region labels in new imaging data, but also the age of the individual (morphological age). Since this enables the explanation of differences in the temporal domain, the authors demonstrated systematic differences along the gestational age axis between control subjects and fetuses suffering from lissencephaly (Fig. 21.3).

Future Challenges in Fetal (f)MRI and Outlook

Fetal fMRI is a powerful approach investigating brain development in utero and has the potential of generating biomarkers for developmental prognosis in the future; the major challenges to focus on can be summed up as follows:

1. Coping with motion artifacts: Motion is a far more relevant nuisance factor in fetal imaging compared to adult imaging. The head of the fetus moves relatively freely, and methods that can cope with or compensate for large head movements are necessary. This is crucial for functional imaging studies, since they rely on data acquired over long periods of time, and are prone to confounds that have to be identified and accounted for when analyzing the data.
2. Improving resolution: Due to motion, the speed of image acquisition is highly important, typically reducing the resolution or field of view of imaging data.
3. Generating accurate labeling: Due to the rapid development of both shape and tissue proper-

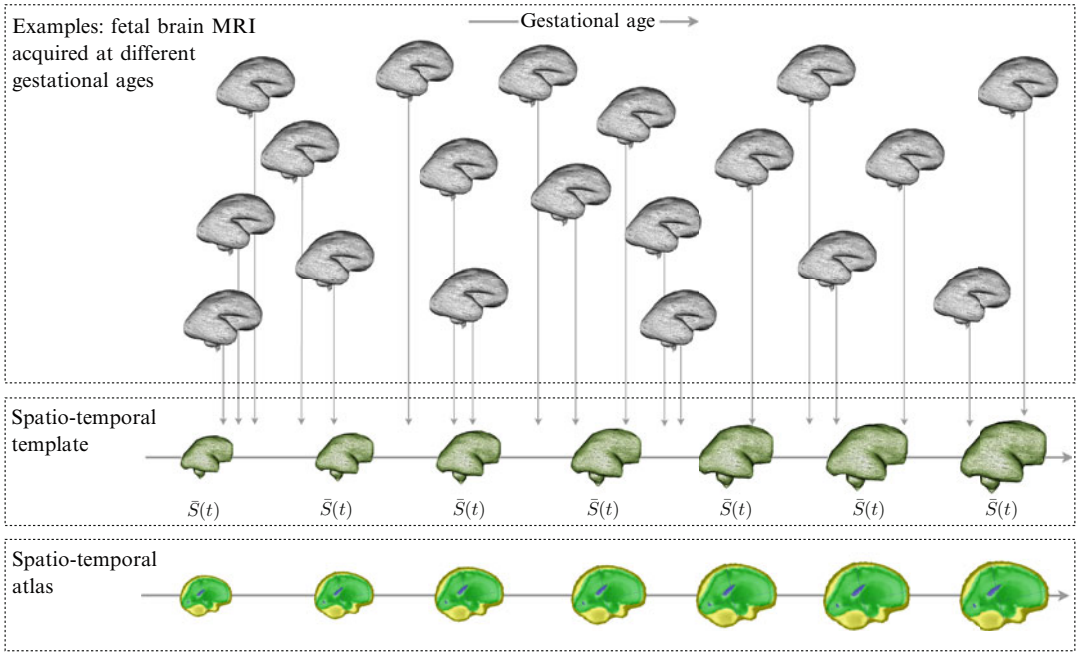

Fig. 21.3 Spatio-temporal fetal atlas. Spatio-temporal atlases are typically a function of gestational age, and are built based on examples that span a representative age range (e.g., Dittrich et al., 2014; Kuklisova-Murgasova et al., 2011). For each age, that atlas can generate a template that represents the typical appearance of the imaging data (e.g., MRI) and a corresponding labeling of brain regions (e.g., CSF, gray matter). This age-specific template and atlas is usually generated by a process that weights the influence of the examples based on the age difference from the target age. Variants include probabilistic labels (Kuklisova-Murgasova et al., 2011), or semi-supervised atlas building (Dittrich et al., 2014)

ties, the accurate segmentation and assignment of brain regions to the imaging data is much more challenging than in adult data. A critical challenge is to accurately and reliably relate these regions to those observed in adults.

4. Identifying structure across time: Rapid changes and emerging or transient structures render the assignment of correspondence across cohorts difficult. Especially in later stages of gestation, the rapid sulcification makes sophisticated methodology necessary that can establish correspondence across individuals and ages.
5. Establishing a bridge between fetal images and infants: Establishing a bridge between in utero imaging and later developmental stages is an important step in understanding continuing brain development.
6. Understanding the development of function: Novel imaging techniques allow for image acquisition with sufficient speed to observe function. After the initial work, we now have to understand the development of function, and its relationship to structural development.
7. Understanding disease: We have to understand the effects of disease that influence individual morphology and the temporal sequence of morphological development.

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Assessing Functional Brain Development in the Uterus with Fetal Magnetoencephalography

22

Jana Muenssinger and Hubert Preissl

Abstract

Functional brain development is a process starting shortly after fertilization and continues throughout the whole human life span. Especially in the fetal period, the evaluation of brain development involves methodological difficulties due to the inaccessibility of the fetal head. Fetal Magnetoencephalography (fMEG) is a non-invasive technique which enables a direct measurement of fetal neuronal activity, and, due to its high temporal resolution, is especially suitable to evaluate its time course. This makes it an important technique to better understand normal and abnormal fetal development.

In the current chapter, fMEG is introduced and challenges and solutions of this technique are discussed. Additionally, an overview of studies using fMEG to investigate brain development by means of auditory and visual evoked responses is presented and discussed. Moreover, the usability of fMEG to determine fetal states and answer clinical research questions is illuminated.

Keywords

Fetal magnetoencephalography • Functional brain development • Auditory perception • Habituation • Neuroimaging

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Fetal Magnetoencephalography

Functional brain development is a process starting shortly after fertilization and continuing over the whole human life span. Although functional brain development between birth and late adulthood already has been well established, research in humans concerning brain function in utero is in its infancy. Extensive research on fetal brain development is important because the basic structure and initial connection pattern of the human brain originates during the prenatal period. In order for the newborn to see, hear, eat, cry, smell, and taste after birth, the human brain already must be highly developed at birth. Prenatal brain development is a plastic and fragile process and can be influenced by environmental factors. Hales and Barker (1992) studied the influence of fetal and neonatal growth on the development of diseases in later life and developed the "thrifty phenotype hypothesis" stating that "the epidemiological associations between poor fetal and infant growth and the subsequent development of type 2 diabetes and the metabolic syndrome result from the effects of poor nutrition in early life, which produces permanent changes in glucose-insulin metabolism" (Hales & Barker, 2001). Additionally to the consequences of early malnutrition on physical health in later life stated by Hales and Barker (2001), environmental influences in the fetal period also can have consequences for the development of multiple cognitive processes. For example, a relationship between maternal elevated stress level during pregnancy and the development of emotional or cognitive problems (e.g., increased risk to develop hyperactivity or attention deficit, anxiety or delay in language development) in the offspring has been shown (Talge, Neal, & Glover, 2007). These processes, which have a long-lasting effect on the offspring's health, are generally summarized under the concept of "fetal programming," stating that some adult diseases have their origins in fetal development (Plagemann, 2004). Also, the prenatal exposure to substances such as lead, alcohol and cocaine has detrimental effects on fetal cognitive development (Jedrychowski et al., 2009; Singer et al., 2008). Studies of fetal programming effects underline the importance of the study of normal fetal development, enabling a better detection of deviations from the normal developmental trajectory. Only an early detection of abnormal brain development provides the opportunity for early therapeutic intervention to attenuate consequences for later life.

However, because the fetal head is covered by amniotic fluid and maternal tissue and is not directly accessible, investigation of brain development has many challenges. Until recently, a direct evaluation of fetal brain development was not possible because suitable imaging tools were unavailable. Therefore, fetal brain development was mostly indirectly evaluated through fetal movement (Groome, Gotlieb, Neely, & Waters, 1993; Kuhlman, Burns, Depp, & Sabbagha, 1988; Shalev, Benett, Megory, Wallace, & Zuckerman, 1989), startle reflexes (Bellieni et al., 2005) and heart rate changes (Leader, Baillie, Martin, Molteno, & Wynchank, 1984) triggered by vibroacoustic stimulation and quantified by the means of ultrasound. While these studies provided important insights into fetal neuronal development, determining which processes were functional was still controversial. The development of brain imaging techniques has enabled the noninvasive evaluation of human fetal brain activity within the uterus, providing new opportunities to more directly gain information concerning brain development.

The current chapter introduces fetal magnetoencephalography (fMEG) as an imaging tool to evaluate fetal brain development and provides an overview of current fMEG research concerning prenatal brain development.

Direct Recording of Fetal Brain Activity Using Fetal Magnetoencephalography

During the last decades, the development of two neuroimaging tools enabled the direct evaluation of fetal brain activity: functional magnetic resonance imaging (fMRI) (Hykin et al., 1999) and fetal magnetoencephalography (fMEG) (Blum,

Saling, & Bauer, 1985). While both techniques enable the detection of fetal brain activity, they have different advantages and disadvantages. fMEG provides a direct measure of neuronal activity, but the fMRI provides an indirect measure of neuronal activity by measuring changes in blood oxygenation during neuronal activation. FMRI has a high spatial but low temporal (roughly seconds) resolution, which makes this technique suitable to localize the source of activity in the human brain. This technique currently is mainly used in clinical settings for high-risk populations due to the exposure of high sound levels and magnetic fields during fMRI measurements. However, recent studies clearly showed the applicability in normal populations as well (Schöpf, Kasprian, Brugger, & Prayer, 2012; Thomason et al., 2013). In contrast, fMEG has a high temporal resolution (milliseconds equivalent to the neuronal activity time scale) enabling the evaluation of the time course of brain activity. Since this technique is noninvasive—fetuses are neither exposed to any machine sound nor to magnetic fields—it is perfectly suited for basic research as well (Preissl, Lowery, & Eswaran, 2004, 2005).

FMEG combines the established technique of magnetoencephalography (MEG) with the special requirements needed for fetal recordings (Preissl, 2005). Currently, only two dedicated fMEG systems—called SARA systems (SQUID Array for Reproductive Assessment)—are available for research use worldwide. The first was installed in Little Rock, Arkansas, USA while the second was installed at the University of Tübingen in 2008 (Fig. 22.1 displays a picture of the fMEG device located at the fMEG Center in Tübingen, Germany). In addition a fetal biomagnetometer was installed at University of Kansas Medical Center, Kansas City, KS, USA, which is mainly used for fetal magnetocardiography (Gustafson, May, Yeh, Million, & Allen, 2012). Besides these dedicated systems there are several general-purpose systems installed worldwide used for fetal clinical and research purposes (Porcaro et al., 2006; Schneider, Schleussner, Haueisen, Nowak, & Seewald, 2001; Wakai, Leuthold, & Martin, 1996).

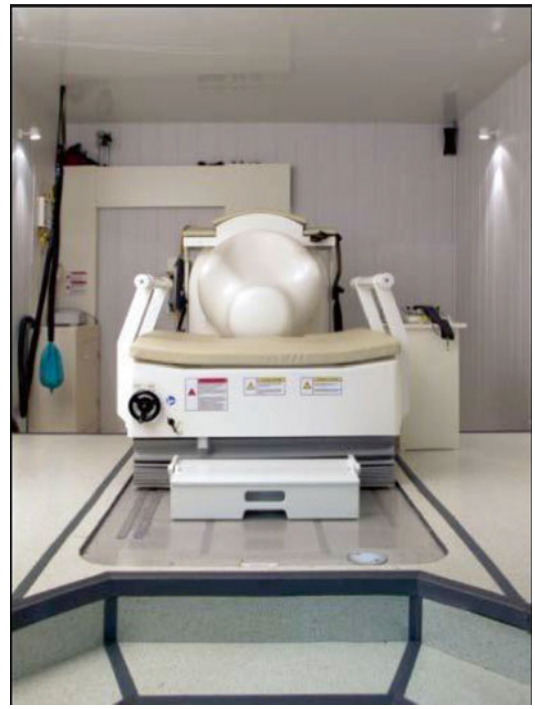

Fig. 22.1 SARA II System (SQUID Array for Reproductive Assessment, VSM MedTech Ltd., Port Coquitlam, Canada) installed at the fMEG Center in Tübingen

Current fMEG research focuses on the development of fetal brain activity elicited by auditory stimulation (auditory evoked responses, AER) or visual stimulation (visual evoked responses, VER). Moreover, fMEG also enables the evaluation of spontaneous fetal brain activity (Eswaran et al., 2007).

Electrical activity of neurons in the fetal brain generate magnetic signals which can be recorded outside of the skull and on the maternal abdomen, because magnetic fields are not distorted by different tissue characteristics. Based on the low magnetic signal amplitudes (femto-picoTesla) only low temperature superconducting quantum interference devices are capable of recording these signals. To enable the measurement of fetal brain activity, the sensors are uniformly distributed in a shell shaped structure matching the form of the maternal abdomen (see Fig. 22.1). For fetal measurements, the pregnant woman sits on the device leaning forward with the maternal

abdomen resting on the sensor array. Four localization coils are attached to confine the maternal position as well as the position of the fetal head in relation to the sensors: one is attached to the maternal right side, one to the maternal left side and the third to the maternal spine. The forth localization coil is attached directly on the maternal abdomen above the fetal head. The position of the fetal head is determined by an ultrasound observation.

The fMEG device installed in Tuebingen includes 156 primary as well as 29 reference sensors (SARA II, VSM MedTech Ltd., Port Coquitlam, Canada). To attenuate magnetic activity from the surrounding environment, the device is installed in a magnetically shielded room (Vakuumschmelze, Hanau, Germany).

During auditory stimulation, sound is produced by a speaker outside the shielded room and travels through air-filled tubes to an air-filled balloon, which is directly placed on the maternal abdomen above the fetal head. Visual stimulation is produced by a panel of light emitting electrodes, which also is placed directly on the maternal abdomen above the fetal head (Wilson, Adams, Murphy, Eswaran, & Preissl, 2009). Due to the fact that fetuses are surrounded by amniotic fluid and exposed to intrauterine "background noise," the sound pressure level of fetal stimulation has to be adjusted to ensure that the sound reaches the fetus. Multiple studies concerning the attenuation level during fetal auditory stimulation have been conducted (Hepper & Shahidullah, 1994; Querleu, Renard, Versyp, Paris-Delrue, & Crepin, 1988; Walker, Grimwade, & Wood, 1971). Because of differing measurement methods and measurement time points (before labor, during labor) results differ among studies. However, in general, external sounds of above 60–65 dB were found to reach the human fetus (Querleu et al., 1988) and all studies agree that higher frequencies are attenuated more strongly than lower frequencies. The sound pressure attenuation of complex external sounds through maternal tissue is expected to be around 30 dB on average.

Additionally to fetal measurements, the fMEG measures neonatal brain activity. Therefore, a cradle is attached to the device, which enables the neonate to lie comfortably and rest its head on the sensor array. For auditory stimulation, the neonate lies on one side with the temporal lobe above the senor array. Auditory stimulation is applied to the contralateral ear. Similar to fetal measurements, sound is produced by a speaker outside the shielded room and is led through air-filled tubes to special headphones, developed for neonatal measurements. For visual stimulation, neonates lay on their back with the occipital lobe above the sensor array. Visual stimulation is presented using a panel of light emitting electrodes, which is placed at approximately 1 m above the fetal head. All neonatal measurements are conducted while neonates are sleeping or lying quietly. During the process, one parent accompanies the neonate inside the shielded room.

Recording and Analysis of Fetal Brain Activity Recorded with fMEG: Challenges and Solutions

In fMEG measurements, the fetal head is separated from the sensory array through maternal tissue and amniotic fluid, which involves challenges for data recording and analysis. To avoid fetal movement in the amniotic fluid during data recording, two precautionary measures are applied. Firstly, ultrasound observations are performed directly before and after the measurement to detect changes in the position of the fetal head and body. Only measurements without major changes in the fetal position are eligible for data analysis. Moreover, in most studies only fetuses in the last trimester of pregnancy are included. During this time period, most fetuses are positioned with their heads in a relatively stable position in the pelvis, which makes large movements unlikely.

Concerning data analysis, it has to be taken into account that fetal and maternal heartbeats generate magnetic fields, which are much higher (several picoTesla) than the weak magnetic fields of fetal brain activity (several femtoTesla). For fetal and maternal heartbeat attenuation, different techniques are applied, but it is necessary to evaluate the techniques for their suitability for fMEG detection (McCubbin et al., 2006). To eliminate maternal muscle or movement artifacts

occurring randomly, auditory and visual stimulation paradigms are designed in a way, that stimulation is repeated with a high number of trials. Averaging these high numbers of trials causes attenuation of random artifacts and improves signal to noise ratio. Moreover, data analysis is restricted to channels from the sensor area above the fetal head to ensure that only fetal brain activity is analyzed. Further evidence for the validity of these approaches is based on inverse solution techniques, which allow the extraction of the sources generating the signals recorded on the abdomen. It was shown that evoked and spontaneous fetal brain activity corresponds to neuronal generators located in the fetal head (McCubbin et al., 2007; Micheli et al., 2010; Vrba et al., 2004, 2007).

Using fMEG to Evaluate Fetal Brain Development by the Means of Auditory Evoked Potentials

Studies using auditory stimulation to evaluate the development of fetal brain activity presume a functional maturation of the auditory system. This can be expected around the 20th week of gestation, when first auditory experiences are anticipated. Using vibroacoustic stimulation, first fetal behavioral responses were detected between 24 and 25 weeks of gestational age (GA), with stable responses across the group at 28 weeks GA (Birnholz & Benacerraf, 1983). Fetal behavioral reactions to pure tones of different frequencies were detected as early as in the 19th week of GA with stable reactions at 27 weeks GA for 250 and 500 Hz tones and at 33 weeks of GA for 3000 Hz tones (Hepper & Shahidullah, 1994). In accordance with these behavioral findings, auditory evoked responses (AERs) were detected reliably at an age around 28 weeks of GA (Eswaran et al., 2002, 2002; Lengle, Chen, & Wakai, 2001; Schleussner & Schneider, 2004). Following fetuses between the 27th and 40th week of GA, a decrement of response latencies with increasing GA has been detected (Holst et al., 2005; Schleussner & Schneider, 2004). Since faster brain reactions indicate further developed brain mechanisms, the reported decrement of response latencies can be interpreted as evidence for continuous brain maturation during that time period.

To further evaluate the functional development of the auditory system additional studies were performed to determine the auditory discrimination capacity of fetuses. In an oddball paradigm including 500 Hz tones as standard tones and 750 Hz tones as deviant tones to fetuses between 33 and 36 weeks GA, Draganova et al. (2005) showed mismatch negativity (MMN) responses as an indicator of the ability to discriminate stimuli (Näätänen, 1992) in 48 % of the evaluated fetuses. These results could be improved in a follow-up study investigating sound discrimination in fetuses between 28 and 39 weeks GA as well as in neonates (Draganova, Eswaran, Murphy, Lowery, & Preissl, 2007), showing MMN responses in 66 % of the fetuses and in 89 % of the neonates. Similar results for change detection in fetuses and neonates also were found by other groups (Huotilainen et al., 2003; Huotilainen et al. 2005). These studies demonstrate that fetuses are able to discriminate between different pure tone stimuli in the last trimester.

However, besides pure tone discrimination, several studies examined whether fetuses and neonates are able to differentiate between pure and complex tones (Kushnerenko et al., 2007; Muenssinger et al., 2013). Results revealed that neonates have higher response amplitudes to complex sounds such as white noise than to pure tones. In a first study, novel sounds were used as complex stimuli (Kushnerenko, Ceponiene, Balan, Fellman, & Näätänen, 2002). However, to ensure that the higher response amplitudes were not caused by the novelty of the sounds per se but by their complexity, in a second step, white noise composed of several different sound frequencies was used as complex stimuli. Also in this paradigm, neonates showed enhanced response amplitudes for the complex stimuli, indicating that a differentiation between complex sound stimuli and pure tones independent of stimulus novelty is possible shortly after birth (Kushnerenko et al., 2007; Muenssinger, Matuz, Schleger, Draganova et al., 2013). A similar paradigm as well as a simplified paradigm to assess auditory change detection between pure and complex tones also was presented to fetuses in the last

trimester of pregnancy (Muenssinger, Matuz, Schleger, Draganova et al., 2013). In this group, results showed no difference between response amplitudes to pure or complex sounds. This might indicate that the fetal brain in the last trimester of pregnancy is not yet mature enough to discriminate between pure and complex sounds. However, this conclusion must be carefully evaluated because fetal measurements involve difficulties, which are not present in neonatal recordings. While the human ear is designed to function in an air-filled environment, the fetal ear is surrounded by and filled with amniotic fluid and separated from the sound source by maternal tissue (Gerhardt et al., 1996). Therefore, sound perception is expected to be different in the fetus than in the neonate. Using microphones or hydrophones to evaluate sound attenuation in utero, it was shown that high frequencies are strongly reduced by the maternal abdomen and the amniotic fluid while low frequencies can be enhanced (Hepper & Shahidullah, 1994; Querleu et al., 1988; Richards, Frentzen, Gerhardt, McCann, & Abrams, 1992). Therefore, it can be expected that the broad range of frequencies combined in the white noise stimuli did not reach the fetuses with an equal sound pressure level, compared to the neonatal measurements. Therefore, the perceived difference between the pure tone and the white noise stimulus might have been much smaller in fetuses than in neonates, which could explain the missing signs for sound discrimination in fetuses. However, further research is needed to clarify this theory.

Another important step in fetal brain development is the ability to learn. One basic form of fetal learning is habituation, the response decrement to repetitively presented stimulation. In a recent study, Muenssinger et al., (2013) evaluated the response decrement to repetitively presented auditory stimulation in fetuses and neonates. After an initial response sensitization, a response increment evaluated between tones one and two, response decrement for the preceding tones was found. This response course with an initial sensitization followed by a response decrement fits the criteria of habituation according to the revised version of Thompson and Spencer's (1966) criteria of habituation (Rankin et al., 2009). In their paper, criteria to differentiate between habituation and sensory fatigue as cause for response decrement are discussed and evaluated (for a detailed overview please see Rankin et al., 2009). However, to more thoroughly differentiate between habituation and sensory fatigue as cause for the evaluated response decrement, a dishabituator—a simple tone differing in tone frequency—was inserted into the array of repeated tones (Fig. 22.2I shows the paradigm used by Muenssinger, Matuz, Schleger, Draganova et al., 2013, Muenssinger, Matuz, Schleger, Kiefer-Schmidt et al., 2013). It was shown that response decrement was specific to the frequency of the repeatedly presented tone, response increment was seen for the dishabituator (stimulus specificity) (for results please see Fig. 22.2IIB). Moreover, MMN responses between the dishabituator and the last tone before the dishabituator could be found in around half of the fetuses, indicating that change detection was possible in parts of the group. Stimulus specificity as well as the occurrence of MMN responses are strong indicators that response decrement was due to habituation and not caused by sensory fatigue. If sensory fatigue had been the cause for response decrement, a generalization of response decrement including the dishabituator would have been expected, because both tones activate similar neurons in the brain and no MMN responses would have been expected. Dishabituation as another indicator for habituation could not be found in the paradigm. Therefore, even though the study showed strong indicators for habituation as reason for response decrement, further research is needed to thoroughly solve the question concerning the cause for response decrement. While the development of simple forms of learning as habituation is an important prerequisite for focused attention in later life, another important question is if the ability of numerosity discrimination is congenital or has to be learned postnatally. Schleger et al. (2014) investigated that question by presenting an oddball paradigm with auditory stimuli differing in numerosity to 30 fetuses in the last trimester of pregnancy as well as to 30 neonates and a control group of 14 adults. MMN responses were calculated as indicators for the ability to discriminate

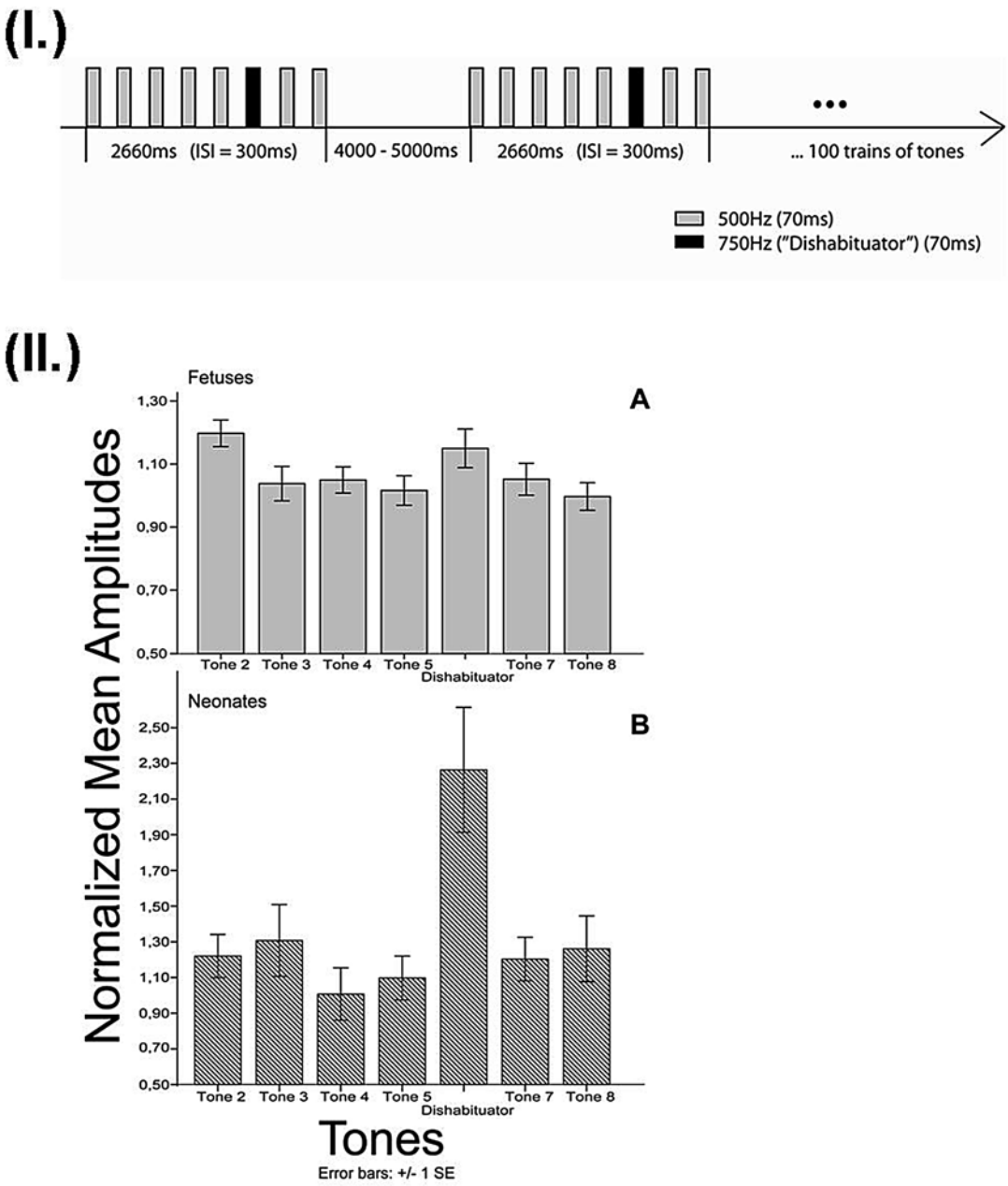

Fig. 22.2 (**I**) Paradigm to investigate habituation in fetuses and neonates. A dishabituation stimulus was inserted to differentiate between habituation and sensory fatigue as cause of response decrement. (**II**) Normalized amplitudes of tones 2–8 of fetuses (M(age) = 34.9 weeks GA) (*A*) and neonates (M(age) = 36.4 days) (*B*) presented with the auditory habituation paradigm. Mean and standard error are displayed (Figure adapted from Muenssinger, Matuz, Schleger, Kiefer-Schmidt et al., 2013)

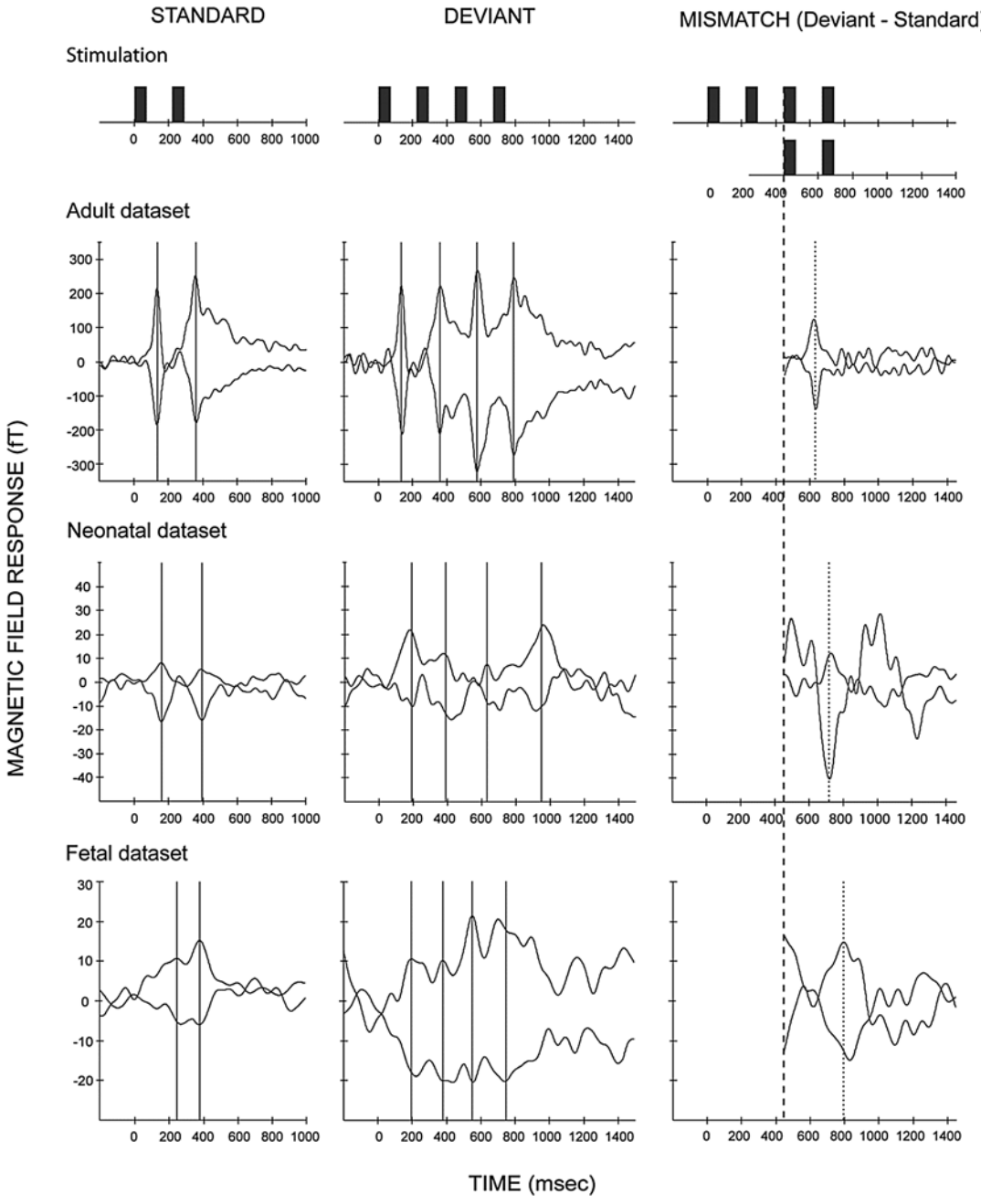

Fig. 22.3 Paradigm used to assess numerosity (*top line*) as well as examples for adult, neonatal (M(age) = 38.8 days) and fetal (M(age) = 35 weeks GA) data (Schleger et al., 2014)

between the differing stimuli consisting of either two-tone beeps (standard) or four-tone beeps (deviant). Results showed mismatch responses in 100 % of the adult group, 100 % of the neonatal group and in 74 % of the fetal group, indicating that numerosity discrimination is already present in the last trimester of pregnancy (for results please see Fig. 22.3).

Using fMEG to Evaluate Fetal Brain Development by the Means of Visual Evoked Potentials

Similar to the auditory system, parts of the visual system mature throughout the late prenatal period. While the cone receptors of the human eye, important for photopic vision, need visual input and develop during the first months of neonatal life, the rod receptors, important for scotopic vision, develop and mature completely without the influence of light. They develop during the late period of fetal life and are functional around term (Graven & Browne, 2008).

Using fMEG, the first fetal brain reactions to the presentation of light flashes have been detected as early as 28 weeks GA (Eswaran, Preissl et al., 2002; Eswaran, Wilson et al., 2002). In a follow-up study, Eswaran, Lowery, Wilson, Murphy, and Preissl (2004) evaluated the development of the visual system. To do so, fetuses between 28 and 40 weeks GA were presented with light flashes and visual evoked responses (VERs) were recorded. Developmental progress could be shown in the response detection rate as well as in response latencies. While in the age group between 28 and 32 weeks GA, a response detection rate of 60 % was observed, the response detection rate increased to 70 % for fetuses in the age group between 32 and 36 weeks GA. In a sub-group between 36 and 40 weeks GA, only few fetuses showed VERs (28 %); however, ultrasound analysis in this sub-group revealed that the position of most nonresponders was with the eyes to the maternal back. Therefore, it can be assumed that visual stimulation did not reach the eyes of those fetuses, explaining the missing brain response. Concerning response latencies, a clear decrement in response latencies could be shown with increasing GA. These studies underline the suitability of fMEG to directly investigate fetal VEPs and its development over gestation.

Habituation as a basic form of learning also has been investigated in the visual domain. Sheridan et al. (2008) presented trains of four light flashes to fetuses between the ages of 29 and 37 weeks GA as well as to infants between the age of 6 and 22 days. While a clear response decrement between flashes one and four was detected in the group of newborns, the response rate in the group of fetuses was rather low. VERs could only be detected in 29 % of this group. This low response rate could be explained by the low signal-to-noise ratio for visual measurements. If the response to the first flash is close to the noise level, the responses to repeatedly presented visual stimulation may become undetectable. However, analysis of the datasets of the responding fetuses showed a decrement of VER amplitudes between the first and second flashes in this group. Similar results also were found by Matuz et al. (2012), who added an auditory dishabituator after a train of four light flashes to differentiate whether the response decrement was due to habituation or a consequence of sensory fatigue. In a group of newborns, a response decrement between light flashes one and four as well as response recovery to the auditory stimulus could be detected. In accordance with the prior study (Sheridan et al., 2008), detection rate in the fetal group also was low with response decrement from the first to the second light flash in the responding fetuses. However, response recovery to the dishabituator in the group of newborns serves as an indicator that response decrement was due to habituation and was not likely caused by sensory fatigue.

Taken together, these studies show that VERs can be detected in the last trimester of pregnancy. In contrast to auditory stimulation, which reaches the fetus independent of its position in the maternal abdomen, visual measurements are dependent on the fetal eye position.

Using fMEG to Determine Fetal Behavioral States

Fetal and neonatal behavioral states were first described by Nijhuis, Prechtl, Martin, and Bots (1982). The authors showed that it is possible to define four distinct behavioral states after 36–38 weeks of gestation. The states are sleep and wakefulness and both states can be further subdivided into quiet and active. The determination of

the different states in the original definition is based on heart rate, heart rate variability, fetal body and eye movements. It was shown that the fetal behavioral states can be extracted from fMEG measurements by means of an actocardiogram including heart rate, heart rate variability and fetal movement. Initial evidence showed that fetal behavioral states affect brain activity (Haddad et al., 2011) and recent studies substantiated this by showing that response latencies to auditory stimuli depend on the fetal behavioral state (Kiefer-Schmidt et al., 2013). This clearly indicates that future studies in fetal development should take into account the fetal state and possible changes in fetal states during investigations. In addition it was shown that the time of the day when a measurement was performed did not affect the state and response latencies (Sonanini et al., 2014).

Using fMEG for Clinical Research Questions

Currently, the main goal of fMEG research is to gain more information and understanding of healthy brain development during the prenatal period. Only understanding the normal developmental trajectory allows an early detection of any deviations, which is needed for an efficient and goal-directed therapy. During the last few years, fMEG also was used to assess more clinical research questions. As described before, brain development in the prenatal period is a fragile process, which can be influenced by many environmental factors. One compromising factor for fetal brain development is intrauterine growth restriction (IUGR). Due to placental insufficiency, the fetal brain may be oxygen-deprived which in turn increases the risk for developmental delays or neurologic damage. To evaluate this question, Kiefer et al. (2008) presented pure tone bursts to a group of fetuses who were small for gestational age (SGA, weight below the tenth percentile of the age group) as well as to a group of healthy fetuses ≥27 GA respectively. In the group of SGA fetuses, Doppler scans could validate placental insufficiency in 12 of 14 fetuses. To determine differences in fetal development, AER latencies were compared between both groups. As expected, results showed increased AER latencies in the group of SGA fetuses compared to healthy fetuses of the same age group, indicating delayed brain development in fetuses from pregnancies with placental insufficiency.

Also, a first magnetoencephalographic study concerning the influence of maternal metabolic changes on fetal brain reactions has been conducted (Linder et al., 2014). Investigation of the AERs of 13 healthy fetuses whose mothers underwent an oral glucose tolerance test (OGTT) revealed a relationship between maternal insulin levels and fetal brain responses. Fetuses from mothers with low insulin sensitivity showed slower brain responses to the presentation of pure tones (results are displayed in Fig. 22.4). These findings provide the first important evidence for fetal programming of central insulin resistance measured with fMEG.

Another factor influencing fetal brain development has been shown to be substances administered to pregnant mothers (Jedrychowski et al., 2009; Springer et al., 2010). However, clinical conditions sometimes require the administration of medication to the mother. One such clinical requirement is the administration of steroids to accelerate fetal lung maturation if premature birth is expected. However, animal studies provided the first evidence that steroids also are associated with delayed myelination and fetal brain growth (Whitelaw & Thoresen, 2000). Based on this finding, Schneider et al. (2011) used magnetoencephalography to evaluate a group of fetuses whose mothers were given steroids for clinical reasons. AERs to pure tone stimulation were evaluated before steroids were administered as well as not later than 3 h after steroid administration. Results showed a delay in AER latencies after steroid administration in comparison to the latencies before steroid administration, indicating that steroids are beneficial for the maturation of the fetal lung, but simultaneously influence fetal brain development.

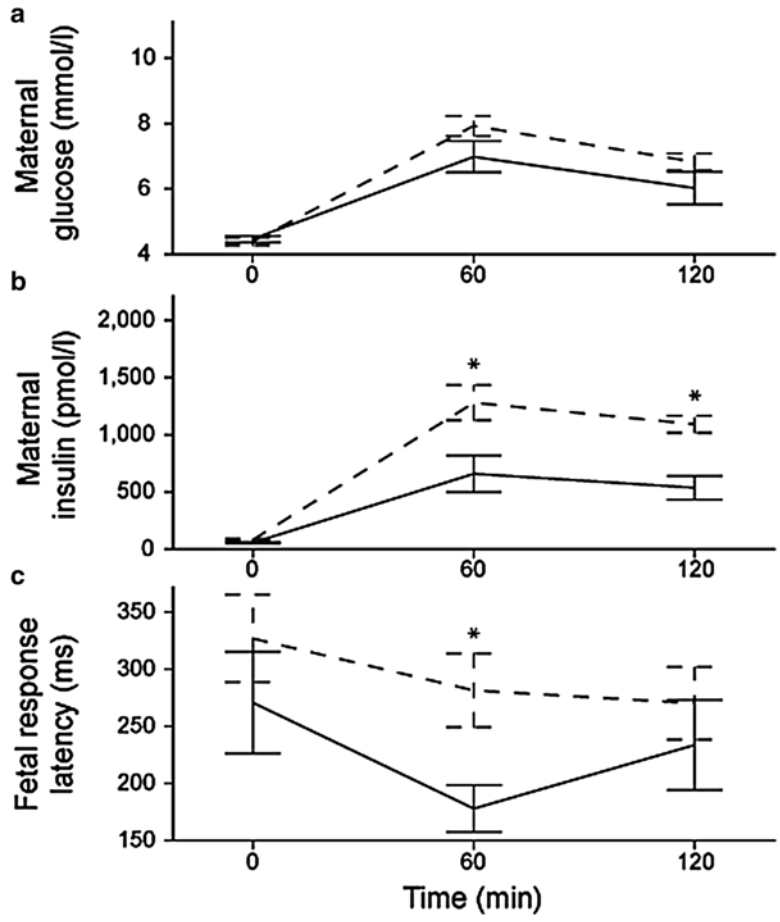

Fig. 22.4 Maternal glucose levels (**a**), maternal insulin levels (**b**) and fetal (*M* (age = 30.9 weeks GA) response latencies (**c**) during OGTT in insulin-resistant ($n = 6$, *dashed line*) and insulin-sensitive women ($n = 7$, *solid line*). Data are shown as mean ± SEM; $*p < 0.05$ between groups (Linder et al., 2014)

Summary

In summary, research on prenatal brain development has experienced major progress during the last few decades because of the development of imaging technologies in general and fMEG in particular. While only an indirect evaluation of brain development using behavioral measures was possible before, the development of brain imaging techniques suitable for fetal research enabled direct recordings of fetal brain responses. Even though fetal investigations involve many difficulties based on factors which are not easily controllable, like movement of the fetus and low signal to noise ratios of the signals, aggregating findings over multiple fMEG studies, researchers have been able to uncover fundamental fetal brain responses and their development over the course of gestation such as auditory change detection, numerosity discrimination or habituation. Importantly, future research using fMEG in high-risk pregnancies could have the potential to detect atypical development earlier and, therefore, enable faster and/or more goal-directed intervention/treatment.

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23 Fetal Assessment Using Biomagnetometry: Neurobehaviors, Cardiac Autonomic Control, and Research Applications

Kathleen M. Gustafson and Elena Anda Popescu

Abstract

Fetal biomagnetometry offers an unprecedented opportunity to study the longitudinal development of the human fetus in a noninvasive manner. Magnetic fields emanating from the electrical currents of the fetal body pass through the maternal body with little distortion, making it possible to distinguish electrophysiologic activity of the heart, diaphragmatic movements associated with hiccup and breathing movements, and oromotor activity associated with nonnutritive suck and swallow. Our research interest is focused on developmental origins and in that regard, we have found fetal biomagnetometry to be superior when measuring the effects of interventions on the development of fetal neurobehaviors and cardiac autonomic control. In this chapter, we first describe our experience characterizing specific fetal neurobehaviors and their associated magnetic field properties along with the effect on cardiac autonomic control. Next, we describe our findings in two separate research studies. The first was designed to determine the impact of maternal physical activity during pregnancy on fetal and infant cardiac autonomic control. The second describes the results of a clinical trial that was designed to determine the effect of docosahexaenoic acid (DHA), supplemented to pregnant women during the last two trimesters of pregnancy, on fetal autonomic development. Finally, we discuss the need for a comprehensive analytical approach

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that takes into account the complex integration of physiologic systems in the developing fetus. We hope that this work serves as a foundation for future studies that will determine how the fetus adapts as an individual to the in utero environment.

Keywords
Biomagnetometry • Magnetocardiology • Developmental programming • Fetal • Autonomic nervous system • Central nervous system • Heart rate variability • Pregnancy

Introduction

The use of fetal cardiac signals as a method for fetal surveillance has been in practice as early as the 1600s (Gultekin-Zootzmann, 1975). Clinical surveillance is primarily concerned with fetal well-being during gestation and adequate oxygenation during delivery. However, the monitoring of fetal cardiac signals and movements also provides a window into the behavioral development of the fetus (DiPietro, Bornstein, Hahn, Costigan, & Achy-Brou, 2007; DiPietro, Hodgson, Costigan, Hilton, & Johnson, 1996b; Van Leeuwen, Cysarz, Edelhauser, & Gronemeyer, 2013).

The concept behind developmental programming is that the in utero environment influences fetal organ and brain development, thereby altering offspring physiology and metabolism, which can then set the stage for future health (Barker, 1994; Barker et al., 1993). Historically, researchers have demonstrated a link between birth weight and health or disease as an adult (Cheung, Low, Osmond, Barker, & Karlberg, 2000; Osmond & Barker, 2000; Phillips, Borthwick, Stein, & Taylor, 1996; Stein et al., 1996). Although birth weight is easily and routinely obtained, it is not a sensitive indicator of fetal neurodevelopment. Programming effects can impact both ends of the fetal growth spectrum, i.e., maternal undernutrition can lead to a small for gestational age fetus (Langley-Evans, 2001), while maternal obesity and gestational diabetes increases the risk for a large for gestational age fetus (Armitage, Poston, & Taylor, 2008; Vasudevan, Renfrew, & McGuire, 2011). While on opposite ends of the fetal growth spectrum, both outcomes result in greater risk for obesity and metabolic disease in the offspring. This limits the use of birth weight as a sensitive indicator for developmental programming effects and, further, does not explain the neural mechanisms behind associations with health or disease. With the current knowledge and growing evidence of developmental programming effects comes a need for methods that are sensitive enough to detect physiological differences between groups (e.g., maternal factors) and research interventions. As such, our research has focused on the use of biomagnetometry.

Since the recording of the first fetal magnetocardiogram (MCG) in 1974 (Kariniemi, Ahopelto, Karp, & Katila, 1974), fetal biomagnetometry has proven to be sensitive with respect to recording simultaneous maternal–fetal cardiac signals. Magnetic fields emanating from the electrical currents of the fetal heart pass through the maternal body with little distortion so that precise MCG recordings of singleton and even twin pregnancies are readily obtained without loss of signal (Burghoff & Van Leeuwen, 2004; Comani et al., 2005; Mensah-Brown, Lutter, Comani, Strasburger, & Wakai, 2011). Historically, the early work of other investigators was largely focused on the use of MCG for detection of fetal arrhythmias and conduction defects (Ishii, Chiba, Sasaki, Kawamata, & Miyashita, 2003; Kandori et al., 2001, 2003; Li, Strasburger, Cuneo, Gotteiner, & Wakai, 2004; Menendez et al., 1998, 2000; Van Leeuwen, Lange, Bettermann, Gronemeyer, & Hatzmann, 1999). As the field evolved, longitudinal studies of rate, variability,

and conduction time intervals emerged that further defined the developmental time course of cardiac autonomic control and growth (Anastasiadis et al., 1999; Kotini et al., 2001; Rassi & Lewis, 1995; Schneider et al., 2008; Van Leeuwen et al., 1999, 2004; Van Leeuwen, Geue, Lange, Hatzmann, & Gronemeyer, 2003; Van Leeuwen, Lange, Klein, Geue, & Gronemeyer, 2004; Verklan, Padhye, & Brazdeikis, 2006). The field was further enhanced by efforts to characterize the influence of fetal state on heart rate variability (HRV) (Lange et al., 2009; Zhao & Wakai, 2002). The most recent investigations expand the field by describing periods of maternal–fetal heart rate (HR) synchrony (Van Leeuwen et al., 2003, 2009, 2014), the use of fetal HRV metrics to calculate a fetal "brain-age score" (Hoyer et al., 2013), and the individuality of HRV (Van Leeuwen et al., 2013). The latter is particularly important because, if the individuality of HRV is embedded, as suggested by Van Leeuwen et al., then this would have important implications for the use of fetal HRV as a proxy for developmental programming effects.

In order to accurately measure HRV, each R-peak of the cardiac signal (QRS complex) must be clearly identified. To that end, biomagnetometry excels over other imaging technologies. The additional use of independent component analysis (ICA) to map multivariate maternal and fetal data into temporally independent components allows for the clear separation of various biomagnetic signals. These independent components can then be identified with respect to their temporal and spatial characteristics and subsequently reconstructed to their source waveform [i.e., maternal MCG, fetal MCG, fetal diaphragmatic magnetomyogram (dMMG), fetal nonnutritive suck (NNS), and fetal hiccups].

This chapter details our experience with a dedicated fetal biomagnetometer. We describe the use of several metrics of HRV applied to the fetal MCG and three characteristic fetal movements known to modulate HR. We then demonstrate the research application of these methods in two longitudinal studies in which maternal factors may influence the development of offspring HRV.

Fetal Magnetocardiography: Methods

Maternal–fetal recordings were obtained by using an investigational 83-channel fetal biomagnetometer custom-designed by CTF Systems (a subsidiary of VSM MedTech, Ltd). This device is housed in a magnetically shielded room (Vakuumschmelze AK3b, Hanau) to reduce interference from external environmental magnetic fields. The spatial distribution of the sensor array is designed to cover the gravid maternal abdomen (Fig. 23.1).

Weak magnetic fields are measured outside the maternal abdomen with extremely sensitive sensors (axial gradiometers) that use superconducting quantum interference devices (Hamalainen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). The use of fetal magnetometry for in utero assessment offers the advantage of multichannel recordings with millisecond time resolution. When compared to electrographic recordings, the quality of biomagnetic recordings is superior, especially during the last trimester of pregnancy when the *vernix caseosa* covering the fetus hampers electrical conductivity. Further, when compared to Doppler ultrasonography, biomagnetometry has three distinct advantages: (1) the recordings are completely noninvasive, (2) fetal biomagnetic fields other than MCG are

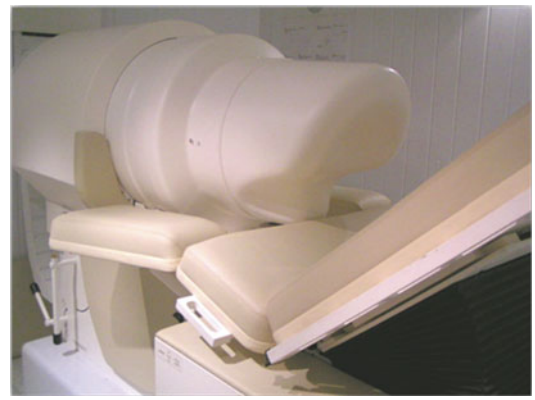

Fig. 23.1 Dedicated fetal biomagnetometer with maternal support. Women are seated in a slightly reclined position with legs on both sides of the biomagnetometer interface. The concave surface of the sensor array overlays the gravid maternal abdomen

detectable, and (3) MCG captures the QRS complex so that each R-peak can be precisely identified; critical for the accurate calculation of HRV.

Women are seated comfortably in front of the biomagnetometer in a slightly reclined position. There is minimal contact with the concave surface of the sensor array, without putting pressure on the maternal abdomen. Legs and arms are supported and a head rest is used to ensure maternal comfort for longer recordings. Recordings typically last 18–36 min, durations that have been well tolerated by the women in our studies.

Data are acquired using a 300 Hz sampling rate with a recording filter of 0–75 Hz. Subsequent digital filtering (bidirectional fourth-order Butterworth filters) between 0.5 and 40 Hz is applied offline. As seen in Fig. 23.2a raw data are a mix of maternal and fetal signals. While maternal and fetal MCG are detectable in the raw data, there are instances where peaks overlap, making the identification of every R-peak difficult. Therefore, after filtering, the data are presented to an Infomax ICA algorithm implemented in EEGLAB (Delorme & Makeig, 2004) in order to segregate the contributions from spatially distinct electrophysiologic sources (e.g., maternal heart, fetal heart, fetal diaphragm). The algorithm is based on the maximum entropy method developed by Bell and Sejnowski (1995) with the natural gradient feature introduced by Amari, Cichocki, and Yang (1996). ICA estimates an optimal linear transformation that maps the multivariate data into a series of temporally independent components with distinct spatial distribution. ICA has proven to be efficient for artifact rejection in biomedical signal applications but in particular for fetal cardiac signal reconstruction (Comani, Mantini, Alleva, Di Luzio, & Romani, 2004) from multichannel fetal biomagnetic recordings.

Visual inspection of the ICA components clearly shows maternal cardiac activity (green), fetal cardiac activity (blue), fetal breathing activity (red), and a yet undefined component (orange) with their corresponding field maps Fig. 23.2b, c. Maternal and fetal fiducial QRS peaks are automatically detected using a template-matching algorithm developed by our team. False positive and false negative detections are manually corrected and abnormal (e.g., ectopic) beats are removed from the analysis. The instantaneous *R-R* interval or interbeat interval (IBI) sequence is then converted to a time-series by linear interpolation at 10 Hz, resulting in a signal that is appropriate for frequency analysis (IBI time-series). Metrics of cardiac rate and time-domain metrics of HRV are derived from the *R-R* intervals while metrics in the frequency domain are derived from the interpolated IBI time-series. We use a variety of analytic methods for HRV analysis, such as QRSTool and CMetX (a suite of freely available tools for transforming ECG/MCG data to metrics of HRV) (Allen, Chambers, & Towers, 2007) (http://www.psychofizz.org) and Kubios HRV v2.1 (http://kubios.uku.fi), including in-house developed Matlab (MathWorks, Inc., Natick, MAs) tools. Many of the common metrics are universal between the software packages. Both QRSTool and Kubios allow for the selection of time specific events in the data. This is especially useful when comparing HRV during different fetal states or activities. QRSTool employs a unique measure of frequency band-limited IBI time-series (Log respiratory sinus arrhythmia; Log RSA) and Kubios features several nonlinear HRV variables. The most common HRV metrics used in our analyses of fetal HRV are as follows:

1. Metrics of cardiac rate (mean IBI, mean HR) which are influenced by both parasympathetic and sympathetic activity.
2. Time-domain metrics summarizing overall (both short-term and long-term) HRV, influenced by both parasympathetic and sympathetic activity, which include:
   (a) The Standard Deviation of Normal to Normal interbeat intervals (SDNN).
   (b) The natural log of the variance in the IBI time-series (log HRV).
   (c) Mean absolute successive IBI difference (Mean Successive Difference; MSD).
3. A time-domain metric of short-term HRV; the Root Mean Square of Successive Differences (RMSSD).

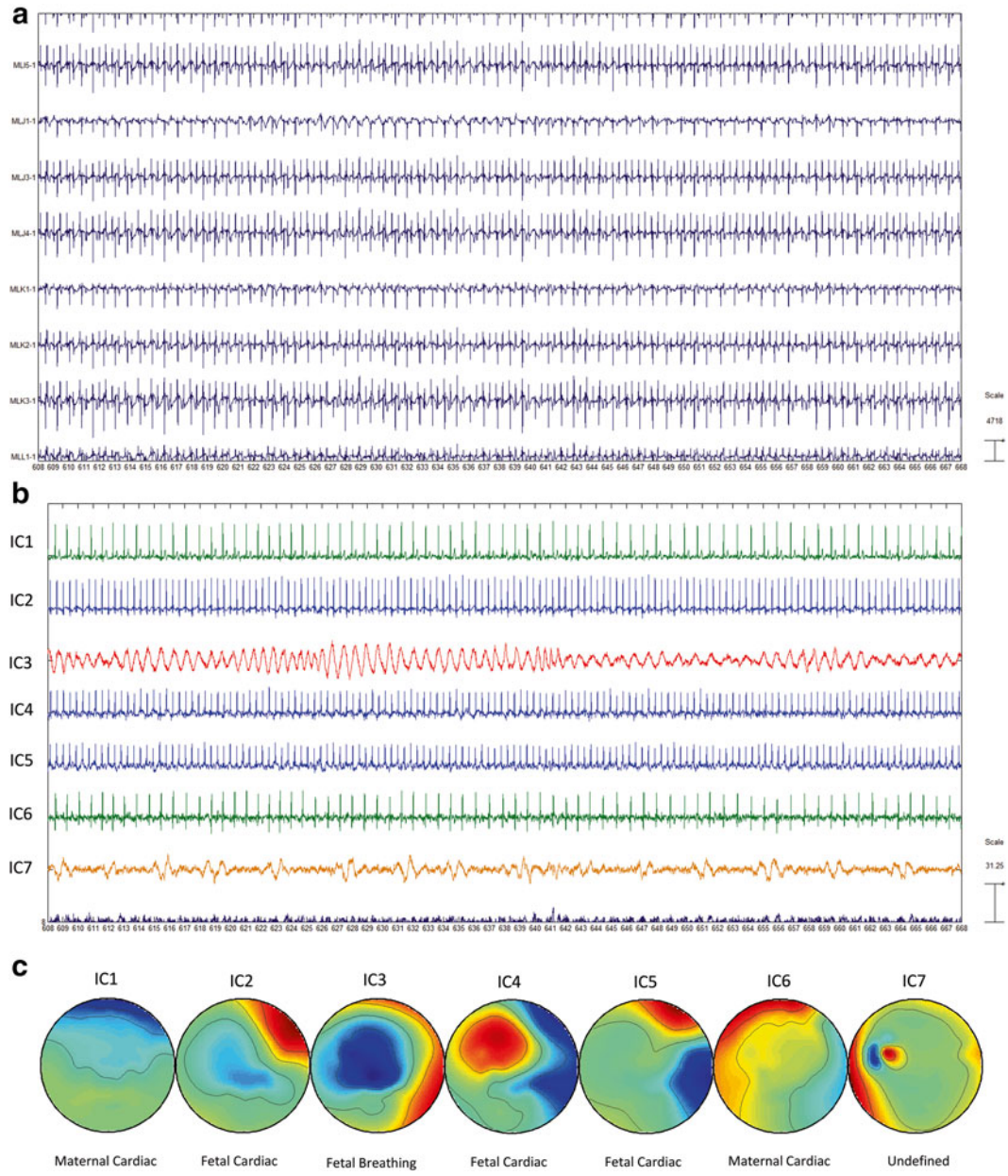

Fig. 23.2 Channel data, independent components (IC), and field maps corresponding to each component. (**a**) Sixty seconds of channel data are shown for seven of the 83 channels. Channel data are a mix of maternal and fetal signals. One can distinguish maternal cardiac activity, fetal cardiac activity, and fetal breathing movements in the raw data. (**b**) Following ICA decomposition, maternal and fetal cardiac signals are clearly differentiated, as are other movements. Data are shown for the same 60-s period shown in the channel data. Maternal cardiac components are seen in IC1 and IC6; fetal cardiac components are seen in IC2, IC4, and IC5. The fetal dMMG, generated by movement of the diaphragm is seen in IC3. In IC7 a common but still undefined signal is seen that may be related to fetal eye movement. (**c**) Field maps corresponding to each component are shown. (The field maps shown correspond to the entire 18-min recording.) After components are identified and attributed to their appropriate source, components can be used to reconstruct the signal of interest and then used to register the data back to channel space

4. A putative parasympathetic metric; the natural log of the variance in the band-limited IBI time-series (log RSA). The band-limited signal is selected to capture the breathing frequency of the fetus (0.4–1.7 Hz) (Allen et al., 2007), and following filtering of the IBI time-series in this band, the natural log of the variance in this filtered time-series yields the metric log RSA.
5. From the IBI time-series, we employ a time–frequency analysis tool implemented in Matlab. The output from time–frequency analysis (power integral of defined frequency bands) is based on the work of David et al., using the following frequency bands: very low frequency (VLF) [0.02–0.08 Hz], low frequency (LF) [0.08–0.2 Hz], intermediate (Int) [0.2–0.4 Hz], high frequency [0.4–1.7 Hz], and total power (TP) [0.02–1.7 Hz] (David, Hirsch, Karin, Toledo, & Akselrod, 2007).

The beat-to-beat variability in HR, indexed either from HR (bpm) or IBI time-series, is an important tool for the assessment of health status (Lieb, Parson, Mamikunian, & Vinik, 2012; Linden, Diehl, & Berlit, 1997; Schroeder et al., 2005; Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012; Thayer, Yamamoto, & Brosschot, 2010; Tsuji et al., 1996), development (David et al., 2007; DiPietro et al., 1996b, 2007; Sahni et al., 2000; Schneider et al., 2008; Van Leeuwen et al., 2013; Van Leeuwen, Geue, Lange, Hatzmann & Gronemeyer, 2003; Wakai, 2004; Zeskind, Goff, & Marshall, 1991) and psychological state (Brosschot, Van Dijk, & Thayer, 2007; Elliot, Payen, Brisswalter, Cury, & Thayer, 2011; Friedman & Thayer, 1998; Hall et al., 2004; Hansen, Johnsen, Thornton, Waage, & Thayer, 2007; Park, Van Bavel, Vasey, Egan, & Thayer, 2012; Thayer & Lane, 2009). Generally speaking, rhythmic changes in HR are a reflection of the complex interplay between the parasympathetic system (activation decreases HR) and the sympathetic system (activation increases HR). However, many other factors can influence HRV including activation of arterial baroreceptors, thermoregulatory control, the renin–angiotensin system and cognitive load. Understanding the influence of these elements may be more relevant to measures of adult HRV. The factors that are more likely to influence fetal HRV are gestational age and behavioral state.

It is well established that differences in fetal state have a significant effect on fetal HRV. Active fetal states are associated with higher HR and HRV while quiet states are associated with lower HR and lower HRV. Therefore, accurate determination of fetal movements and an understanding of their influence on cardiac rhythm are essential for HRV interpretation (Pillai & James, 1990a, 1990b). The historical method for determining fetal state was developed by Nijhuis, Prechtl, Martin, and Bots (1982) using two ultrasound scanners with simultaneous registration of the fetal heart rate pattern (FHRP). State 1F is defined as a quiet state with brief, gross body movements, no eye movements, and is associated with FHRP A, a stable pattern with a small oscillation bandwidth. In State 2F, an active state, there are frequent, periodic gross body movements along with movements of the limbs. Eye movements are observed along with FHRP B, described as a wider oscillation bandwidth with frequent accelerations during movements. State 3F, a quiet state is associated with gross body movements, eye movements, and FHRP C, described as a stable pattern with wider oscillations than those seen in the quiet state, 1F. Finally, State 4F is a highly active state associated with continual fetal movement, trunk rotations, and eye movements. This state is associated with FHRP D, which shows prolonged, high amplitude accelerations.

Because the biomagnetomer can detect minute magnetic fields in the femto-Tesla range, electronic devices near the sensors produce an overwhelming artifact. Therefore, simultaneous ultrasound/MCG recordings are impractical. However, Ulusar et al. (2011) devised a method that was subsequently used to identify fetal breathing movements by simultaneously recording ultrasound and biomagnetometry. While such a method has definite advantages and applications, the labor-intensive method is impractical for routine long-term recordings. Therefore, we and others rely on the FHRP classification described by Schneider et al. (2008), using magnetocardiology, to determine fetal state. These

FHRPs correspond well with states 1–4F described by Nijhuis et al. (1982). FHRP I corresponds to state 1F, a quiet state with a stable heart rate, oscillation<±5 bpm over a period >15 s with a baseline HR which does not exceed 160 bpm. FHRP II corresponds to state 2F, an active state with an unstable fetal HR (<160 bpm), with frequent, brief accelerations of similar duration. FHRP III corresponds to state 4F, an active state with an unstable baseline, with frequent, long-lasting accelerations exceeding 10 bpm from the baseline. Since simultaneous ultrasound is prohibitive, we use fetal MCG actography to document fetal movements associated with activity state. Fetal MCG actography relies on variations in the fetal QRS amplitude that are due to changes in the position and orientation of the fetal heart relative to the sensors due to movement (Lutter & Wakai, 2011). In Fig. 23.3, fetal HRP and actograms are shown for the activity

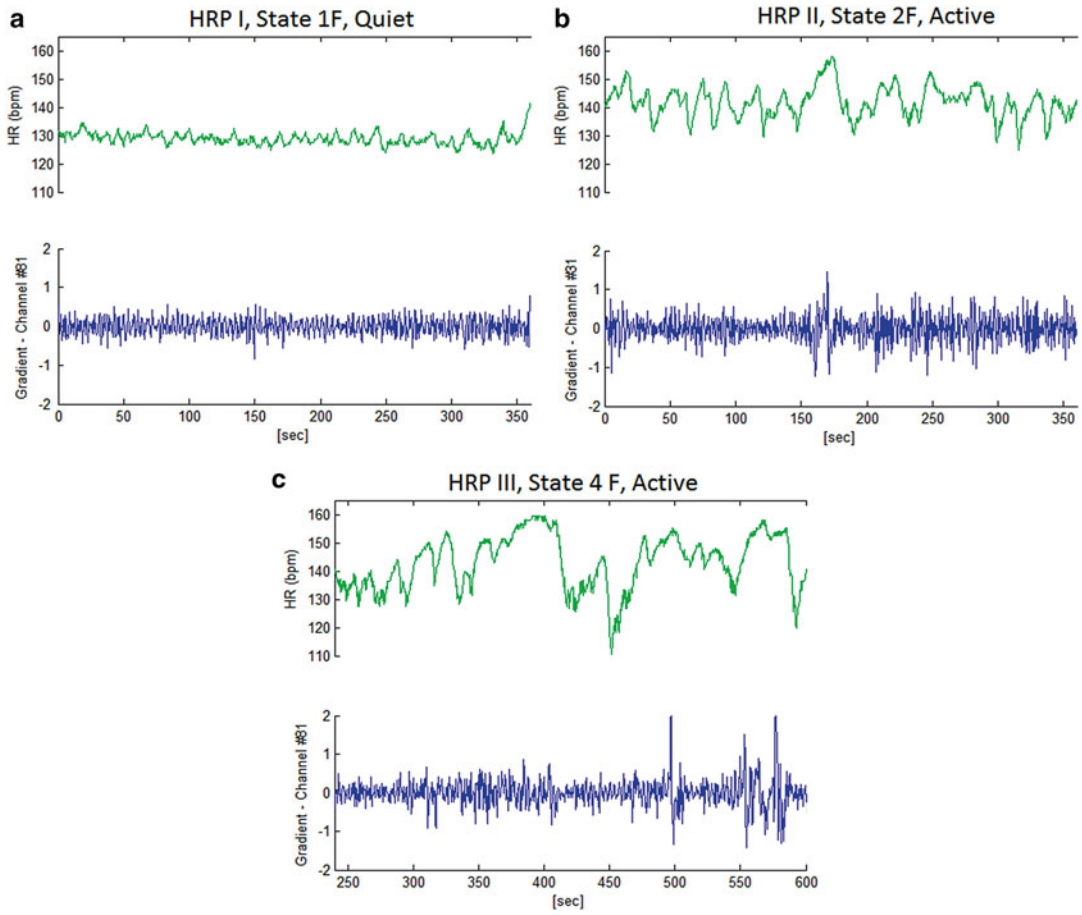

Fig. 23.3 Fetal heart rate patterns (HRP) with corresponding actograms during a 6-min period. (**a**) Fetal HRP I has a stable heart rate with oscillations <±5 beats per minute (bpm). The actogram shows very little deviation from zero, typical of a quiet fetal state. (**b**) Fetal HRP II is an active state with a unstable baseline. There are frequent, brief accelerations of similar duration. The actogram shows several excursions from the baseline, that increase in the latter half of the recording. (c) HRP III is an active state with long duration accelerations >160 bpm. The actogram in the initial phase is similar to that seen in (**b**) but at 500 ms, large excursions from the baseline are seen. These higher amplitude responses are likely due to large body movements. Note that there is not a direct relationship between movement amplitude and HR accelerations. The larger amplitude activity from 500 to 600 ms does not produce an appreciably different HRP from that seen between 250 and 400 ms when the amplitude of movement is smaller

states described above. All records undergo state classification by visual inspection of the fetal HR pattern by two independent experts.

Fetal Heart Rate Variability as a Proxy for Neuronal Integration

During fetal maturation, the functional integration of neuronal systems (brain, heart, motor integration of respiratory and feeding movements) requires increasingly complex coordination. In attempts to characterize the self-organization, adaptation and individual development of the fetus, other investigators have explored nonlinear analysis of HRV (Moraes, Murta, Baffa, Wakai, & Comani, 2012), heart-rate movement coupling (DiPietro, Hodgson, Costigan, Hilton, & Johnson, 1996a), maternal–fetal HR interaction and synchronization (Van Leeuwen et al., 2009; Van Leeuwen, Geue, Lange, Cysarz et al., 2003) and fetal functional brain age using fetal activity state measures combined with various metrics of fetal HRV (Hoyer et al., 2013). A review of these methods and outcomes are beyond the scope of this chapter, but it brings to the forefront how little is known about fetal and newborn behaviors, how the fetus is influenced by biological and environmental factors and how in utero behaviors are related to later life physical and neurological outcomes. Nonetheless, the field of noninvasive fetal surveillance is gaining ground.

The beat-to-beat variability of the fetal heart serves as an index of central to peripheral neural feedback; consequently, fetal HRV is associated with the degree of maturation and integrity of the fetal autonomic nervous system (ANS, Renou, Newman, & Wood, 1969). Fetal heart rate peaks at about 10 weeks of gestation and then decreases as gestation increases. At about 30 weeks of gestational age, increasing influence from the parasympathetic nervous system results in lower HR, greater HRV, and the emergence of distinct HR patterns attributable to fetal activity states (Pillai & James, 1990a). This developmental shift to greater cardiac vagal activity reflects the ability of the ANS to mediate physiological and in utero behavioral and regulatory activity (i.e., body movements, nonnutritive suck, changes in activity states, and periodic breathing movements). The linking of fetal HR accelerations to movement (fetal HR-movement coupling) is a common observation, particularly in the third trimester and is thought to be a sign of central nervous system maturation (i.e., the coordination of autonomic and motor function, DiPietro et al., 1996a).

The integration of autonomic function and fetal behaviors are the earliest precursors to newborn behavioral regulation (Porges & Furman, 2011). For example, HRV serves as an index of attention regulation in newborns (Arditi, Feldman, & Eidelman, 2006), orientation in neonates (Feldman, 2006) and information processing in early life (Bornstein & Suess, 2000). There is good evidence that individual differences in HRV originate before birth and persist from prenatal to postnatal life. This has been shown using longitudinal ultrasound measures (DiPietro et al., 2007; Montenegro, Ramos, Matias, & Barros, 1998) and more recently with MCG using linear and nonlinear measures of HRV (Van Leeuwen et al., 2013). The conclusions of these independent investigators suggest that HRV is engrained during development and exhibits continuity to postnatal life. This has important implications for understanding and measuring developmental programming. It is important to consider metrics of HRV as more than a simple measure of cardiac function but rather, as a proxy for the degree of integration between central and peripheral nervous systems in the developing fetus and a predictor for later developmental outcomes.

Biomagnetic Field Patterns of Fetal Movements

Spontaneous, rhythmic movements such as oromotor (suck, swallow, lip movements), respiratory (fetal diaphragmatic movements) and limb movements occur early in fetal life, before the emergence of complex coordinated behaviors. Neuronal circuits made up of excitatory and inhibitory interneurons, known as central pattern generators (CPGs), generate rhythmic patterns of

activity for coordinated motor output essential for fetal and neonatal life. Studies in animal models have been both elegant and informative; however, the invasive nature of such studies has prevented equally precise measures of electrophysiologic signals in the human fetus. While direct investigation of human fetal CPGs activity in vivo is impossible, with biomagnetometry, we have demonstrated the ability to record and study the output of these networks. Since the application of biomagnetic measurements to prenatal assessment is relatively new, the recorded magnetic field patterns need to be clearly identified and assigned to the appropriate electrophysiological mechanisms. Our goal is to characterize the biomagnetic field patterns associated with specific fetal movements and then to determine how these movements influence cardiac autonomic control and fetal state.

Fetal Nonnutritive Suck

Fetal swallowing, tongue and lip movements have been documented at about 12–14 weeks of gestational age. These individual movements mature to coordinated nonnutritive suck (NNS) by 32 weeks of gestational age, ostensibly preparing the fetus for functional nutritive sucking at birth (Barlow, Burch, Venkatesan, Harold, & Zimmerman, 2012). Nutritive and NNS are distinct in both motor pattern and duration. Postnatal nutritive suck consists of a continuous stream of suck cycles of approximately 1 Hz interrupted by swallowing. This feeding effort requires the simultaneous coordination of suck, swallow, and breathing and is not fully organized in preterm infants until 34 weeks post-conceptual age (Mizuno & Ueda, 2003). In contrast, NNS demonstrates an intermittent bursting pattern of 3–12 suck cycles separated by pauses. The frequency of NNS is about 2 Hz (Finan & Barlow, 1998; Hack, Estabrook, & Robertson, 1985).

NNS has been shown to predict neurodevelopmental outcome at 18 months (Mizuno & Ueda, 2005). Likewise, there appears to be a significant relationship between NNS and the severity of respiratory distress syndrome in preterm infants (Poore, Barlow, Wang, Estep, & Lee, 2008; Stumm et al., 2008), suggesting that NNS might provide insight into central nervous system integrity and motor function in the developing infant (Barlow & Estep, 2006). The development and regulation of NNS also has been shown to provide many other positive benefits including stress reduction, improved oxygen saturation and initiation of nutritive suck, growth, maturation, and improved gastric motility (DiPietro, Cusson, Caughy, & Fox, 1994; Field & Goldson, 1984; Lau, Sheena, Shulman, & Schanler, 1997; Pickler, Frankel, Walsh, & Thompson, 1996). Fetal mouthing and NNS are associated with a distinct, oscillatory fetal heart rate pattern (HRP I, Fig. 23.3) that is most often associated with state 1F.

We have demonstrated that fetal NNS can be recorded outside the maternal body using a biomagnetometer (Popescu, Popescu, Wang, Barlow, & Gustafson, 2008). These data are from a longitudinal study that used fetal MCG recordings for prenatal assessment. Pregnant women underwent a series of recordings between 20 and 38 weeks of gestation. All women provided informed consent and all protocols were approved by the human subject committee at the University of Kansas Medical Center, Kansas City, KS, USA.

Following ICA decomposition of the maternal–fetal MCG, we observed a novel waveform, seen in Fig. 23.4b, c. This waveform had a unique appearance, characterized by a cyclic bursting pattern of 6–12 peaks. The initial peaks were smaller in amplitude with greater inter-peak latency that increased in amplitude approximately one-third through the burst, then decreased in amplitude and latency until termination of the signal. Bursts were separated by several seconds of inactivity. This waveform was identical to that described in the preterm and term infants by Finan and Barlow (1998).

After establishing that the waveform was most likely fetal NNS, we characterized the signals in 34-week and 38-week fetuses (see Fig. 23.5a–c). In order to confirm that the fetal signals were truly NNS, we compared prenatal recordings to postnatal recordings in a newborn at 4 weeks of age, while sucking on a pacifier (Popescu et al., 2008). Suck bursts lasting for 650 s are shown for

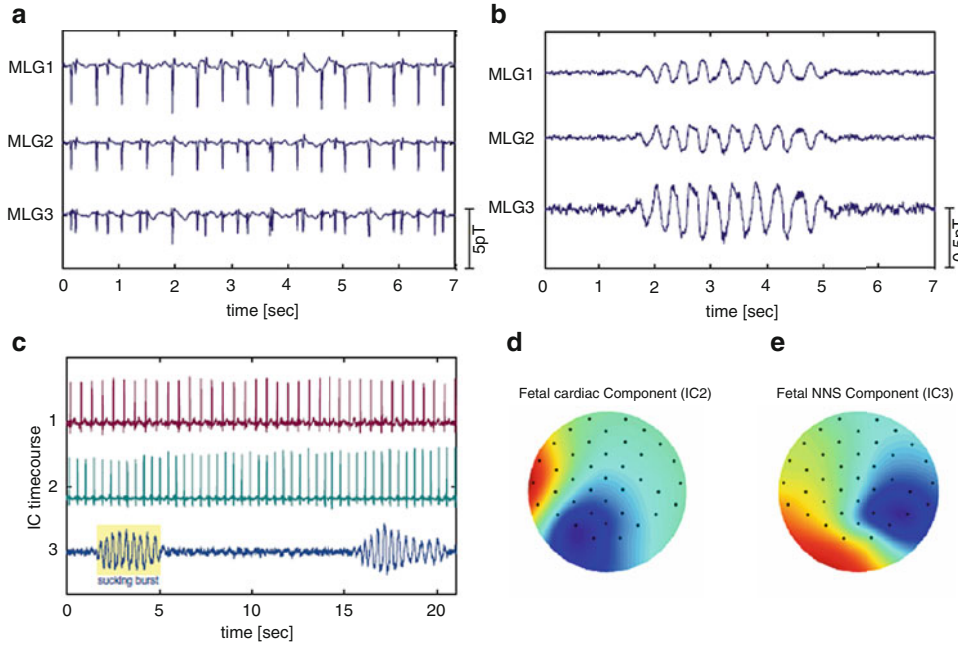

Fig. 23.4 Fetal nonnutritive suck (NNS) in a 38-week fetus. (**a**) Raw biomagnetic data are shown in three channels. The suck burst can be seen in the channels but is obscured by maternal and cardiac activity. (**b**) The data after ICA decomposition shows a typical suck burst with nine cycles. (**c**) Independent components (IC) of maternal cardiac in IC1, fetal cardiac in IC2, and fetal NNS in IC3. (**d**) Topographic field maps corresponding to the fetal cardiac component in IC2 and (**e**) NNS component in IC3

the 34-week fetus in Fig. 23.5a with the resulting fetal HRP below. The suck signal and HRP are overlaid for the activity occurring between 150 and 220 ms. Data for the 38-week fetus are shown in 23.5b. In this case, the suck burst was longer. Note that the trough of the oscillatory HRP occurs 5 s prior to the suck burst and the peak of the HRP occurs 5 s after the initiation of NNS. In 23.5c data from the 4-week-old infant are shown. Infant cardiac components are shown along with two suck bursts, evoked by use of a pacifier. In the overlay, the infant HRP is not as clearly associated with the NNS burst pattern, likely due to the influence of simultaneous eupneic breathing.

The frequency modulation at the onset of the NNS burst was ~3.5 Hz for both fetuses (Fig. 23.6). The frequency modulation decreased with each burst until reaching a plateau of ~2.5 Hz around the seventh–eighth burst. Compared to the 38-week fetus, the 34-week fetus had fewer bursts per minute, longer burst duration, and more suck cycles per burst. We identified heart rate variations associated with the NNS bursts of ~1.5 bpm in the 34-week fetus and ~2.25 bpm in the 38-week fetus, suggesting that NNS-HR modulation may increase with gestational age.

It stands to reason that fetal NNS becomes more organized and integrated with advancing gestation. After term birth, when feeding is increasingly coordinated, NNS may become extraneous. In premature infants, it may maintain both a soothing role and promote further development of nutritive suck, a hypothesis that needs further investigation. Nonetheless, these data confirm that the biomagnetic pattern observed is generated by fetal NNS. The morphology of the signal is consistent with the distinctive rhythmic pattern of bursts separated by rest periods documented in the fetus using Doppler ultrasonography (van Woerden et al., 1988), with a burst rate in the same range as the dominant frequencies reported in near term fetus

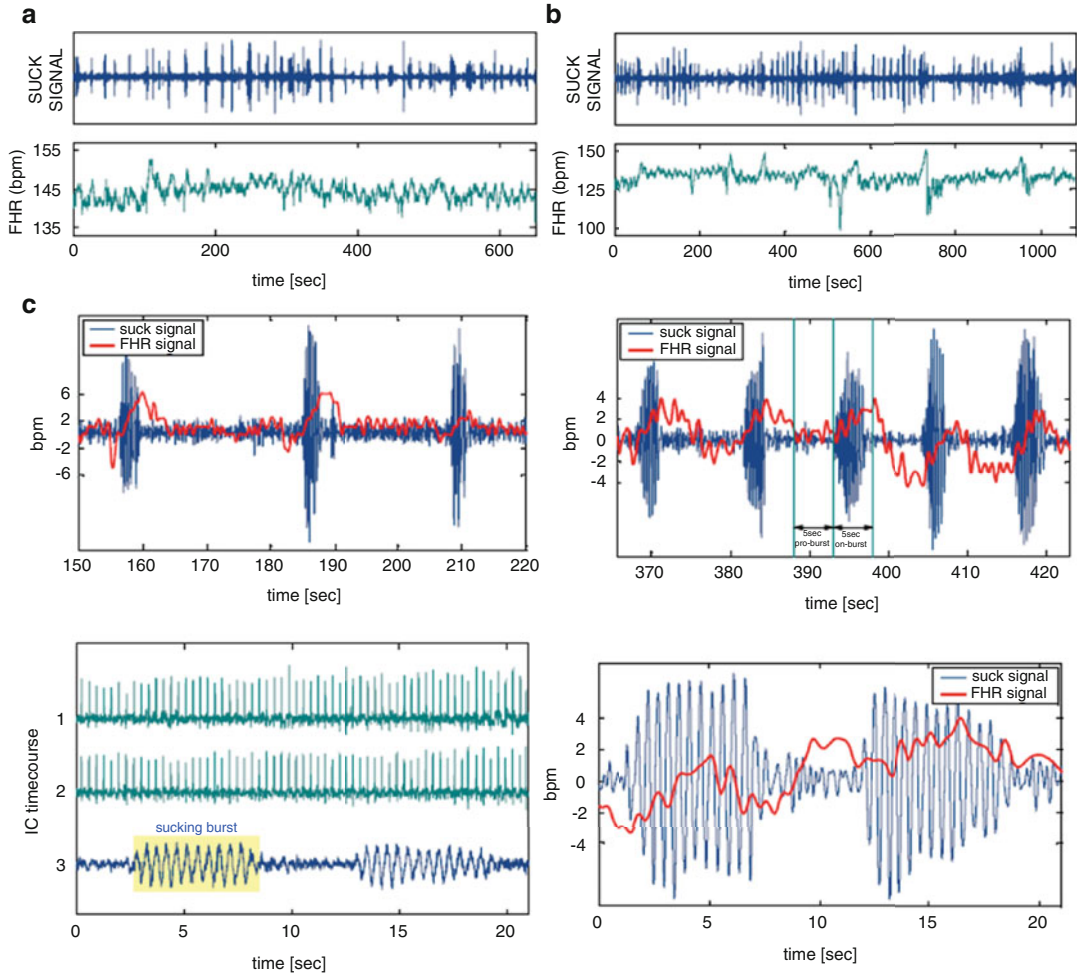

Fig. 23.5 Nonnutritive suck. ICA components associated with NNS are used to reconstruct the signal and put back into channel space. The suck bursts, lasting for 650 ms for the 34-week fetus (**a**) and 1100 ms for the 38-week fetus (**b**) are shown in the *top panels*. The corresponding fetal R-R interval time-series are shown below each suck signal. A section of suck bursts are shown with the fetal HRP overlaid, demonstrating the characteristic oscillatory HRP associated with NNS. Note that the acceleration of HR peaks after initiation of NNS. (**c**) Independent components linked to cardiac activity and suck burst (evoked by a pacifier) are shown for a 4-week-old infant. To the *right*, the suck signal and corresponding HRP are overlaid. Note that, while an oscillatory pattern exists, that the association is not as distinct as that seen during the fetal period. This may be due to the influence of simultaneous eupneic breathing in the infant

(van Woerden, van Geijn, Caron, & Mantel, 1990). This NNS biomagnetic signature also was confirmed by our recording of pacifier-induced NNS in infants. NNS bursts were associated with an oscillatory HR pattern consistent with 1F state-specific fetal HRP I (Nijhuis et al., 1982) that is indicative of regular mouth movements (van Woerden et al., 1988). There was an increase in the mean fetal HR over the whole duration of the burst, consistent with studies using simultaneous recordings of sucking and cardiac activity in full-term newborns (Lipsitt, Reilly, Butcher, & Greenwood, 1976). The intra-burst signal features match the characteristics of the pacifier-induced NNS recorded in infants using measures of suck pressure variation and electromyographic (EMG) recordings (Hafstrom, Lundquist, Lindecrantz, Larsson, & Kjellmer, 1997).

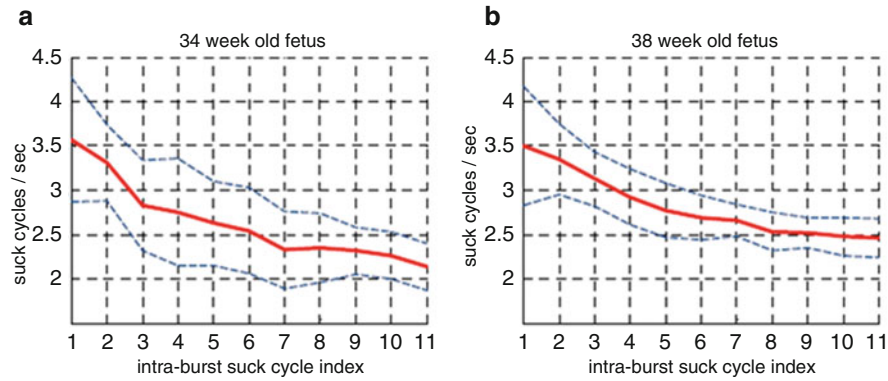

Fig. 23.6 Frequency modulation of NNS. Both the 34-week fetus (**a**) and the 38-week fetus (**b**) initiated suck bursts at around 3.5 Hz. The modulation decreases with each burst and by 7–8 bursts, reaches a plateau at approximately 2.5 Hz. There was more variability in the 34-week fetus with fewer bursts per minute, longer burst duration, and more cycles per burst

Our results confirm that fetal NNS can be recorded in utero using fetal biomagnetometry. More studies are needed to characterize the longitudinal development of NNS from fetal to postnatal life and to understand the interaction with HR. Our observations suggest that NNS becomes more organized with increasing gestational age and that the characteristic oscillatory HR pattern may decrease with advancing postnatal age. Because our research interest has focused on the active fetal states and NNS occurs most often in the quiet state, we have not pursued further evaluation of fetal NNS. Currently, we obtain our recordings 1–2 h after a meal, in the afternoon, to increase the odds of observing the fetus in an active state. One of our current protocols requires women to fast overnight prior to drawing lab samples early in the morning. After the samples are drawn, women are given a multigrain cereal bar with orange juice immediately before recording the MCG. We have found that this increases the chances of observing NNS during the MCG recording as we observe the transition from quiet to active state. Factors to consider for further characterization of fetal NNS are longer recording times, ultrasound recordings prior to MCGs to confirm state and early morning recordings after women have fasted overnight.

Fetal Hiccups and Breathing Movements

Fetal hiccups are one of the first observable fetal movements, appearing as early as 9 weeks postconception (Andonotopo et al., 2005). A hiccup is an involuntary reflex associated with vigorous myoclonic movement of the diaphragm. Prior to 26 weeks of gestation, hiccups are the predominant diaphragmatic movement (Pillai & James, 1990b) and show no relationship to fetal state or movement patterns (van Woerden et al., 1989). Hiccups may appear before fetal breathing movements and may occur separately or concurrently with breathing movements (de Vries, Visser, & Prechtl, 1982). Hiccups also are common in the newborn but diminish with age. Straus et al. (2003) proposed that the CPG responsible for hiccups in humans is a phylogenetic relic of what used to produce ventilation in lower vertebrates (i.e., an archaic gill CPG) and put forth the hypothesis that a CPG that can generate hiccups persists in mammals because it has permitted the development of other CPGs for useful functions (e.g., suckling and eupneic breathing). The hiccup reflex consists of three neural pathways: an afferent pathway via phrenic and vagus nerves; an efferent pathway via phrenic, vagus, cervical,

and thoracic nerves; and a postulated "hiccup center" in the brainstem (Pollack, 2003).

Similar to our observation of NNS, we observed a novel biomagnetic field pattern in two pregnant women who reported feeling hiccups during the MCG recording session. Fetuses were 32 and 34 weeks of gestational age. Hiccups also were recorded in a 3-week-old infant during a follow-up session. After ICA decomposition, we reconstructed the fetal MCG and hiccup signals and examined the modulation effects of fetal hiccups on fetal HR, using the method introduced by Chen et al. (2000). This approach was used because it allows the quantification of transient, temporally localized variations in the RR-interval duration around well-defined events such as fetal hiccups with a higher sensitivity as compared to the global standard measures (in time or frequency domain) used to assess fetal HRV (Popescu et al., 2007).

Raw channel data from the biomagnetometer and ICA components are shown in Fig. 23.7. Fetal hiccups produce two distinct ICA components—a short duration spike of about 30 ms duration and a broad, low-frequency biphasic wave of about 200 ms duration. Visual inspection of the continuous ICA-filtered hiccup data suggests that the low frequency wave has higher hiccup-to-hiccup variability than the initial spiking wave, especially as hiccups decrease and

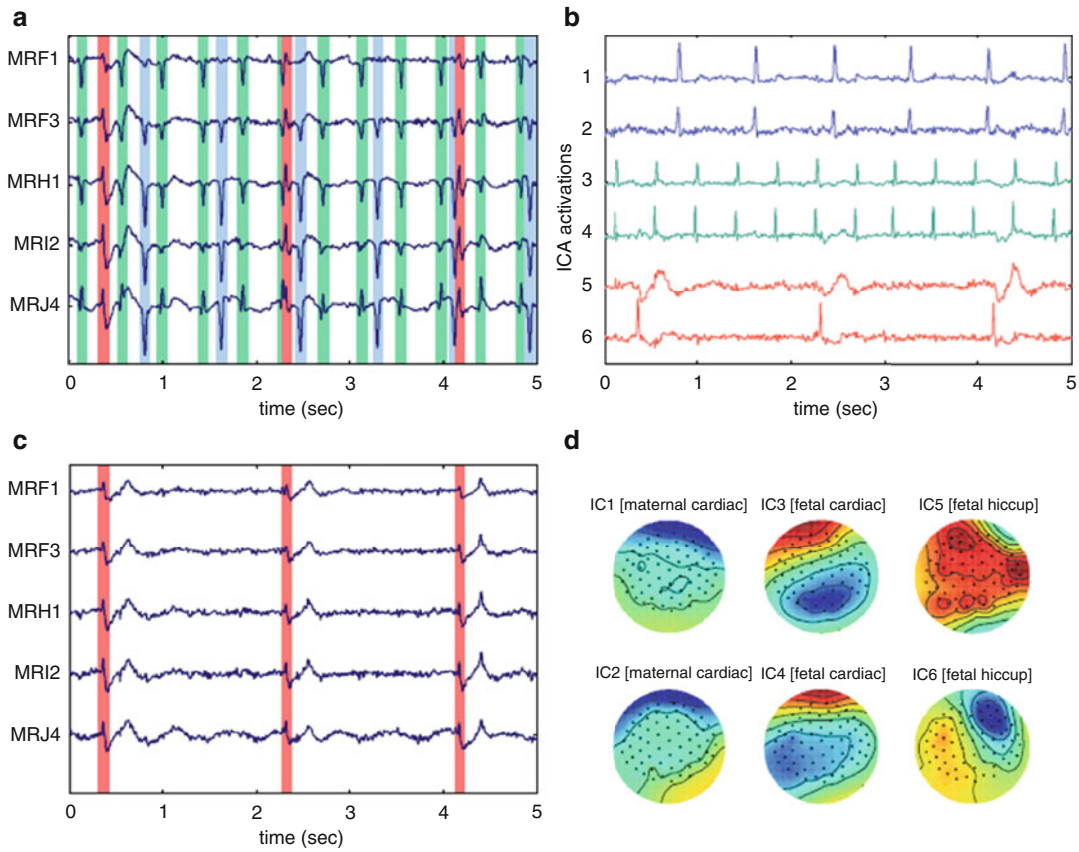

Fig. 23.7 Fetal hiccup. (**a**) A subset of channels showing the raw magnetographic data with maternal MCG highlighted in *blue*, fetal MCG in *green*, and fetal hiccup in *red*. (**b**) Independent components showing maternal cardiac components in *blue*, fetal cardiac components in *green* and the two components associated with fetal hiccup in *red*; a wave in IC5 and a spike in IC6. (**c**) The two components associated with fetal hiccup are used to reconstruct the signal, now seen in channel space. (**d**) Field maps associated with the components; *left*, maternal cardiac in IC1 and IC2; *middle*, fetal cardiac in IC3 and IC4; *right*, fetal hiccup in IC5 (wave) and IC6 (spike)

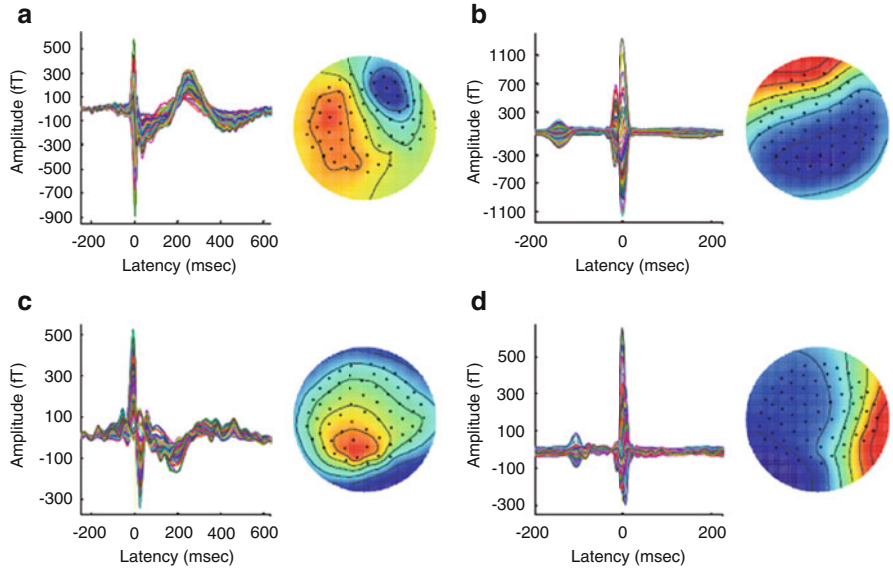

Fig. 23.8 Averaged fetal hiccup waveforms and cardiac signals. Data for two fetuses are shown. The averaged hiccup signals are seen in *panels* (**a**) and (**c**) in contrast with the averaged fetal MCGs seen in (**b**) and (**d**). The magnetic field maps correspond to the spike latency for the hiccup signal and the peak of the QRS signal for the MCG. Note the distinct dipolar field pattern associated with the hiccup and the marked difference from the field distribution for the QRS component

decay. These two components were used to reconstruct the waveform associated with fetal hiccup and the resulting biomagnetic field map shown in Fig. 23.8. For comparison, the reconstructed fetal MCG and field map are also shown. The field maps indicate dipolar sources for both fetal hiccup and MCG that are orthogonal to each other. Data from the infant subject was essentially identical to data from the fetal subjects.

When examining the relationship between hiccups and fetal HR, we found that hiccups induced a rapid modulation of cardiac rhythm marked by an increase in the relative duration of RR-intervals following a hiccup event. The RR-intervals immediately following a hiccup event were significantly longer than the RR-intervals immediately preceding or hosting the hiccup. This suggests an increase in the afferent vagal activity triggered by hiccups, which induces a subsequent increase in the tonic efferent vagal activity. These data are consistent with those of Chen et al. (2000), who observed a similar variation in heart rhythm in association with hiccups.

In contrast to fetal hiccups, fetal breathing movements increase with gestational age. Breathing movements, along with body movements and measures of amniotic fluid volume and fetal HRP, are the mainstays for the clinical assessment of fetal well-being. Breathing and HR are closely intertwined, both functionally and anatomically, through brainstem networks that control autonomic functions critical for survival. Cardiorespiratory coupling is exemplified by respiratory sinus arrhythmia (RSA), a pattern of high frequency HRV associated with the respiratory phase. RSA is characterized by an oscillatory pattern in the HR time-series corresponding to an increase in HR during inspiration and a decrease in HR during expiration. Fetal RSA has been documented in the human fetus during breathing movements, even though the lungs do not participate in gas exchange (Divon et al., 1985; Wakai, Wang, Leuthold, & Martin, 1995). Unlike postnatal eupneic breathing, fetal breathing movements are periodic in nature. Fetal breathing has been observed as early as 11 weeks of gestational age; episodes increase with

gestational age and vary with fetal state, with greater episodes during the active states (Pillai & James, 1990b).

Invasive studies in fetal baboon and lambs best characterized cardiorespiratory coupling during fetal breathing and apneic periods. In baboons, fetal HR was shown to be lower during breathing periods, oscillating about 2 bpm with each fetal breath. Power spectral analysis of the IBI time-series showed increased power in the HF band at the respiratory frequency (1 Hz), associated with greater vagal drive. During apneic periods, fetal HR was higher and power shifted to lower frequency bands associated with greater sympathetic input (Myers, Fifer, Haiken, & Stark, 1990). EMG recordings from the diaphragm of chronically prepared, near-term fetal lambs revealed a periodic oscillating waveform during breathing movements that ceased during apnea (Jansen & Chernick, 1991).

Other investigators who have designed studies to characterize the development and maturation of fetal HRV appreciate the influence of fetal breathing movements on HRV, but methods to unequivocally compare HRV during breathing movements to HRV during apnea have been complicated without the ability to clearly distinguish the activity. David et al. (2007) suggested using a continuous wavelet transform to identify periods of HF power that may be associated with fetal breathing movements and to analyze these sequences separately from those showing an absence of HF power. Presuming that fetal breathing movements are associated with RSA and RSA can be identified by visually inspecting the IBI time-series, Van Leeuwen, Voss, Cysarz, Edelhauser, and Gronemeyer (2011) developed an algorithm that automatically detected fetal RSA and reliably rejected periods where no RSA was present. The procedure processed the IBI time-series in time and frequency domains and identified spectral peaks with characteristics of fetal RSA. Using this method, they were able to detect fetal RSA 96.1 % of the time. However, the high sensitivity came at the cost of mediocre specificity in that all marked episodes had to be visually checked to ensure that false positives would be avoided. This suggests that HF activity in the IBI time-series does not instantaneously appear and disappear at the onset and offset of fetal breathing and that some HF power may be unrelated to fetal breathing movements. Groome, Mooney, Bentz, and Singh (1994) investigated the contribution of fetal RSA to overall HRV spectral power in quiet sleep. They observed a HF peak during fetal breathing that accounted for approximately 20 % of the total power. However, during non-breathing periods, power in the RSA defined frequency band (0.4–1.0 Hz) did not disappear. Likewise, Hirsch, Karin, and Akselrod (1995), using ultrasound and maternal abdominal ECG observed increased HF power during fetal breathing movements, sometimes preceding the breathing movement, or decreasing before the cessation of breathing movements. They also described periodic elevations in HF activity during non-breathing periods. Therefore, identification of fetal breathing episodes based on spectral analysis of HF activity presents many challenges.

The previous work described above challenged us to identify a biomagnetic field pattern associated with fetal breathing movements (Gustafson, Allen, Yeh, & May, 2011). As with NNS and hiccup, we observed an oscillatory signal in the ICA components (see Fig. 23.9) that bore a strong resemblance to the diaphragmatic EMG of fetal lambs (Jansen & Chernick, 1991). Based on the duration and frequency, we hypothesized that the activity was linked to activation of the fetal diaphragm during breathing movements. The signal, hereafter termed "diaphragmatic magnetomyogram" (dMMG), could be observed in raw data as a periodic oscillation of several seconds duration in channels over the fetal mid-section. After ICA decomposition, the response was easily observed and generally ranked within the first ten ICA components. Like fetal hiccups, the magnetic field map was orthogonal to the fetal MCG. Since most of our recordings are obtained during active fetal states, fetal dMMG is the most common fetal motor activity we observe.

Unlike NNS and hiccup that retain the same characteristics after birth, postnatal breathing does not. Postnatal breathing is not periodic, and therefore, recordings of breathing activity in

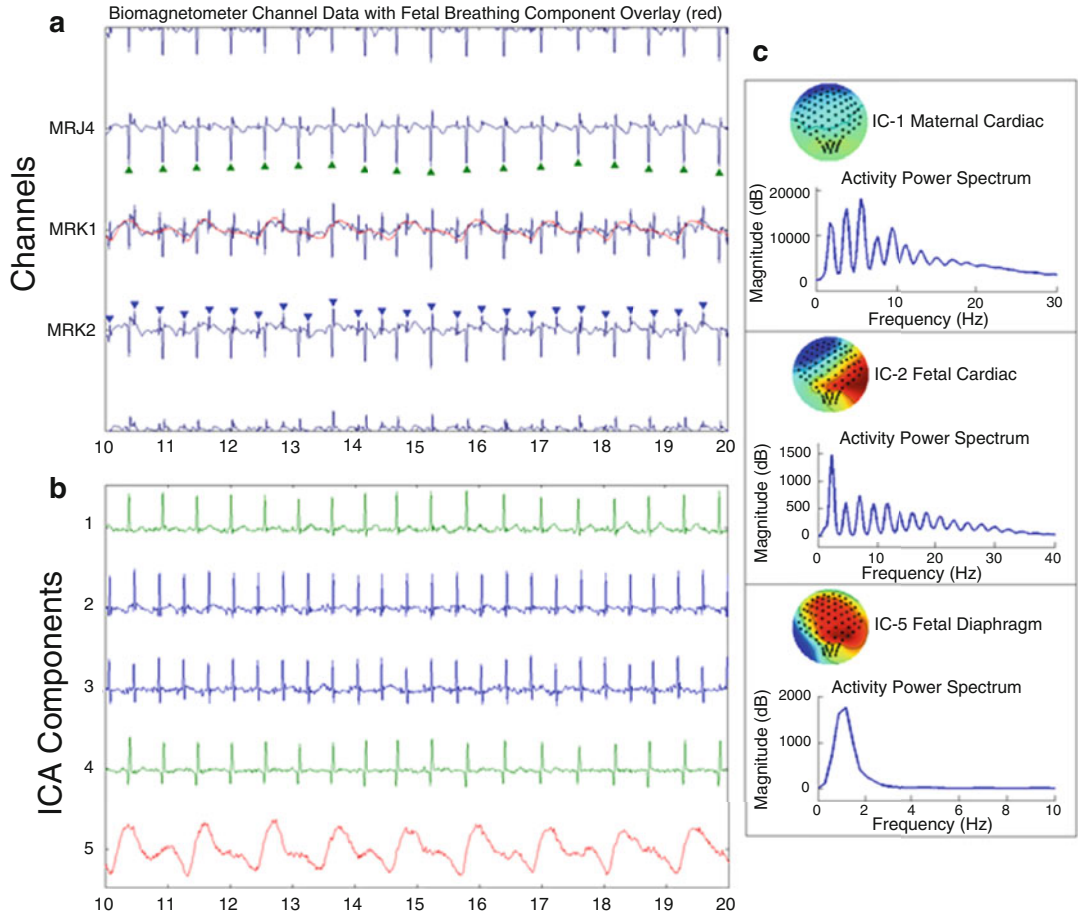

Fig. 23.9 Fetal breathing movements. (**a**) Raw channel data from a subset of channels. Maternal MCG is marked with a *green triangle*, fetal MCG is seen clearly in channel MRK2, marked with a *blue triangle*. In channel MRK1, an oscillating signal is seen, highlighted in *red*. (**b**) After ICA decomposition, maternal (*green*) and fetal (*blue*) cardiac components are clearly observed, as is the oscillating signal (*red*). (**c**) Corresponding magnetic field maps associated with maternal cardiac (*top*), fetal cardiac (*middle*), and fetal diaphragm (*bottom*) activation from fetal breathing movements

infants would not be comparable to fetal breathing movements. We needed to demonstrate unequivocally that the signal we observed was generated by the fetal diaphragm during breathing movements. To establish that the human diaphragm can generate a detectable magnetomyogram during maximal effort, several non-pregnant volunteers practiced paced breathing and breath-holding, deliberating mimicking fetal breathing movements. Once subjects accomplished the task, we recorded the activity in the biomagnetometer.

In Fig. 23.10, we show an example of the adult dMMG at three different paced breathing rates: 1.0, 0.5, and 0.25 Hz. A sinusoidal waveform, linked to the breathing frequency is clearly seen in the channel data. Next, we instructed subjects to breathe at 0.5 Hz for 1 min, hold their breath for 1 min, breathe at 1.0 Hz for 1 min, hold their breath for 1 min, and then to resume eupneic breathing for the last 2 min of the 6 min recording. These results are seen in Fig. 23.11. The results clearly show the recorded dMMG in the middle panel. The effect of paced breathing, apnea and eupneic breathing are obvious in the time–frequency plot, showing increased power at 0.5 Hz and decreased VLF power during this

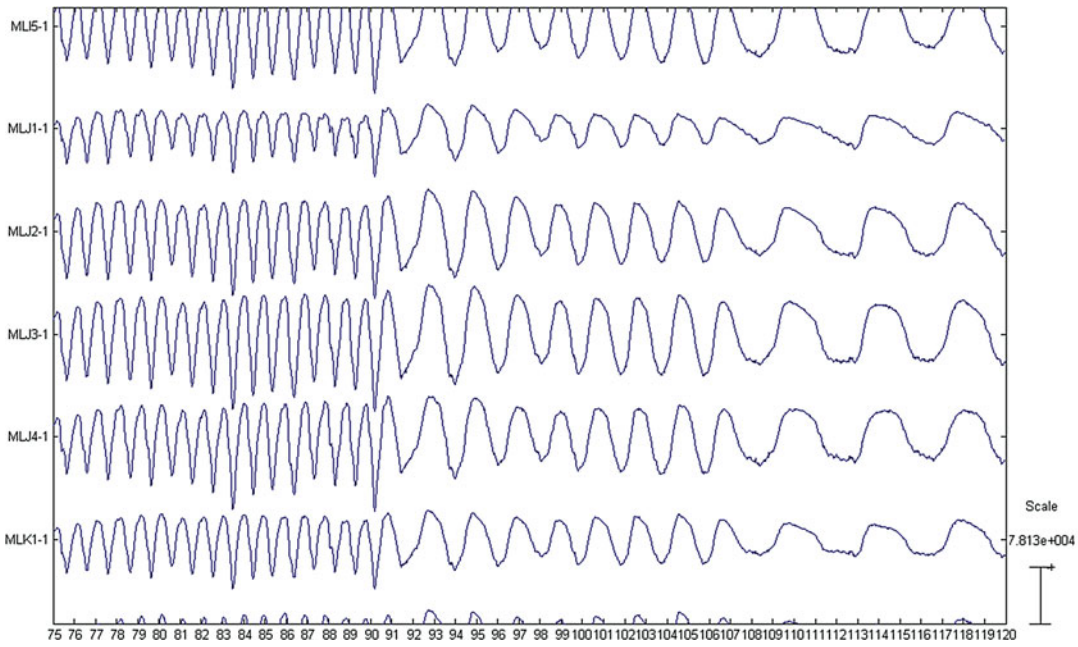

Fig. 23.10 Paced breathing. To demonstrate that the biomagnetometer could record signals generated by movement of the diaphragm, an adult subject practiced paced breathing at 1.0, 0.5, and 0.25 Hz. Data shown are from a subset of channels

episode of paced breathing, with a similar response to 1.0 Hz breathing. During eupneic breathing, the distribution of power normalizes with the greatest power at the breathing frequency (0.125 Hz). RSA is clearly observed in the IBI time-series at the frequency of breathing. During apneic periods in adults, HR decreases.

Next, in a retrospective analysis of 43 individual fetal recordings, we compared the effect of fetal breathing movements on metrics of fetal HR and HRV. We established criterion that the duration of fetal breathing activity had to be equal to or greater than 1 min. Apneic epochs were required to be either immediately preceding or following the breathing epoch and as close to the duration of the breathing epoch without exceeding it. This was done to assure an adequate number of beats, a similar number of beats per epoch (breathing vs. apnea) and to avoid fetal state changes between breathing and apneic epochs. Further, we limited the data pool to fetuses in the active state to avoid comparisons across fetal states. Because we were stringent in our selection, only 20 of the 43 individual fetal records reviewed were selected for analysis. Differences between breathing and non-breathing epochs were compared by the Wilcoxon signed-rank test as the distributions of variables was skewed, even after log transformation; $p<0.05$ was considered significant.

We observed that fetal RSA was clearly identifiable in the IBI time-series (Fig. 23.12). We generated time–frequency plots to visually confirm that HF power in the band corresponding to the onset, rate, and duration of fetal breathing movements. Similar to the findings of Hirsch et al. (1995), it was not uncommon to observe increased cardiac HF power preceding the appearance of organized dMMG activity or the appearance of RSA in the IBI time-series. During short bursts of breathing activity changes in power were less clear, highlighting the pitfalls of determining fetal breathing activity by the appearance of RSA or HF activity.

Fetal HR was significantly lower during breathing epochs (Table 23.1). Short-term HRV (RMSSD

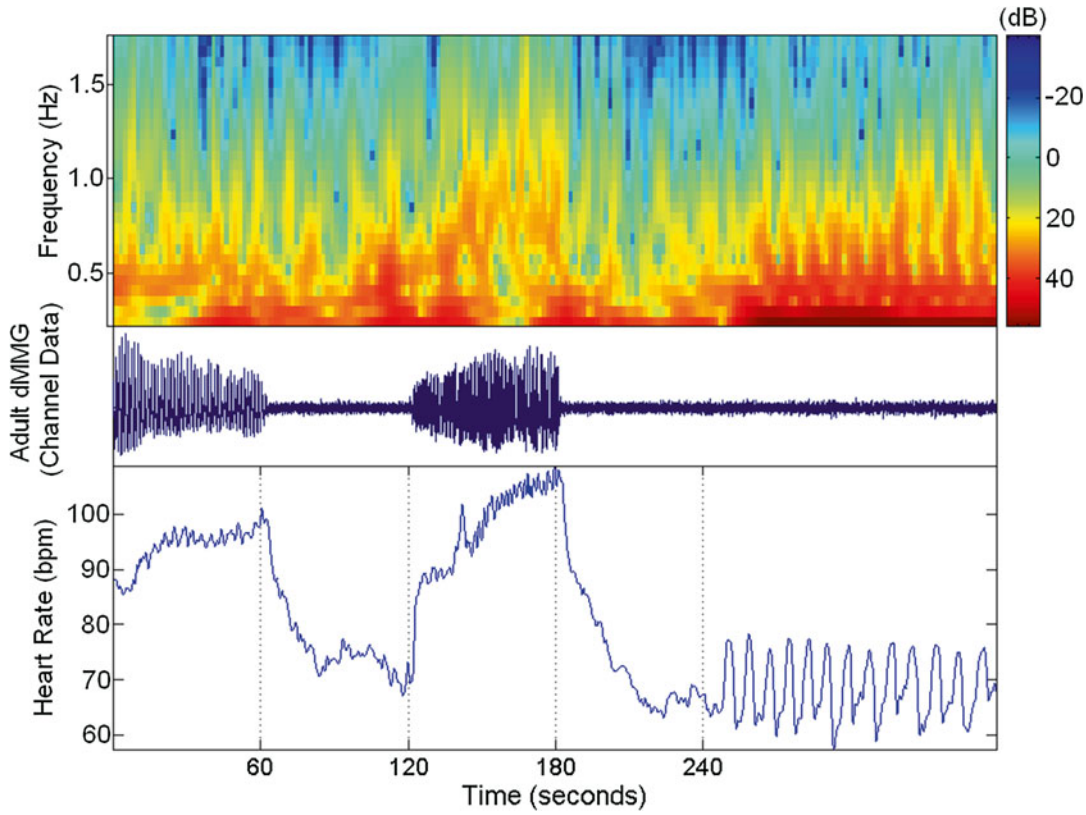

Fig. 23.11 Adult paced breathing, apnea and eupneic breathing. The *top panel* is a time–frequency plot with time on the *X*-axis and frequency on the *Y*-axis. Power (dB) is scaled by *color*. The *middle panel* is the adult dMMG shown for one channel. The IBI time-series is shown in the *bottom panel*. Paced breathing at 0.5 Hz from 0 to 60 s results in a HR acceleration. RSA is seen in the IBI time-series and a corresponding band is seen in the time–frequency plot at 0.5 Hz. During breath holding (apnea) HR decreases to baseline and RSA is diminished. Note however, the activity in the 0.5 Hz range of the time–frequency plot does not disappear, but that power increases in frequencies below 0.5 Hz. From 120 to 180 s, the subject paced breathing at 1.0 Hz. This resulted in further increase in HR, faster RSA, and a distinct band in the time–frequency plot. HR slowly decreased to baseline during the next 60 s of apnea. At 240, after resuming eupneic breathing, HR returns to baseline with the characteristic RSA associated with the slower breathing rate. No dMMG is seen with eupneic breathing. Only exaggerated movements of the diaphragm in adults generate the dMMG

and MSD), a metric of respiratory-related HRV that putatively reflects parasympathetic influences (log RSA), HF power and the frequency band ratios describing sympathovagal balance (VLF/HF and LF/HF) were all significantly higher during breathing epochs. There were no differences in HRV metrics that summarized total HRV (SDNN), Log HRV, total power or power in VLF, LF, or intermediate frequency bands. These results indicate increased parasympathetic input during fetal breathing movements.

Although we could not compare in utero fetal breathing movements to a comparable condition after birth, we are confident that the biomagnetic field pattern described is generated by the fetal diaphragm during fetal breathing movements. As further confirmation, a method of simultaneous ultrasound and biomagnetometry recording was devised by another group. This work provided further evidence that the unique waveform we characterized as fetal dMMG was generated by fetal breathing movements (Ulusar et al., 2011).

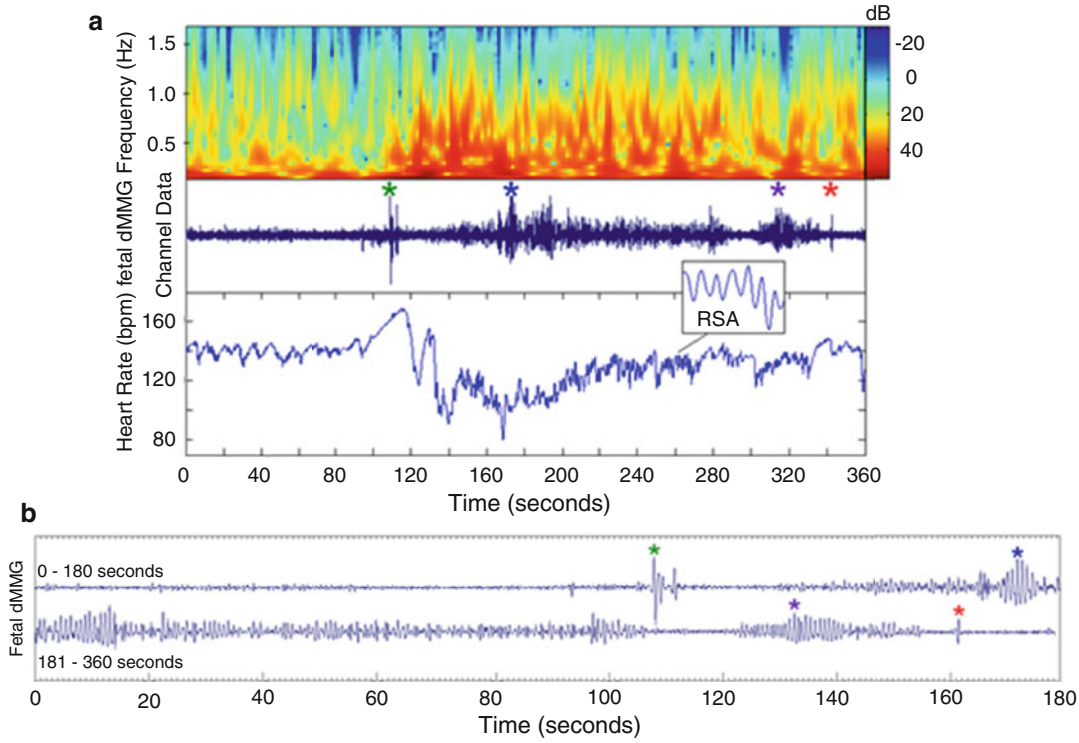

Fig. 23.12 Fetal dMMG. (**a**) Channel data from one representative channel is shown in the *middle panel*. This activity is expanded in (**b**). *Color-coded asterisks* are used to associate activity in the compressed dMMG (*middle panel*) and expanded panel. The fetal IBI time-series is seen below the dMMG. Fetal RSA is apparent in the recording (see *inset*). There is an increase in power, seen in the upper time–frequency plot at the initiation of breathing movements

Table 23.1 Comparison of fetal cardiac metrics during breathing and non-breathing epochs

	Breathing epoch			Non-breathing epoch			
Variable	*Q*1	Median	*Q*3	*Q*1	Median	*Q*3	*p*-Value
IBI (ms)	425.5	437	462.9	383.45	418.75	433.85	0.0001
HR (bpm)	130	138	141	139	143.5	156.5	0.0001
SDNN	8.4	9.9	17.8	10.7	18.0	22.9	0.08
RMSSD	6.3	8.2	9.95	3.65	5.6	7.85	0.006
Log HRV	4.2	4.65	5.7	4.70	5.8	6.2	0.09
MSD	4.85	6.5	7.9	2.55	3.8	5.65	0.0007
Log RSA	1.90	2.25	2.75	0.85	1.25	1.85	0.0021
VLF	1.65	3.63	6.08	2.04	4.91	7.67	0.33
LF	0.77	1.42	2.24	0.67	1.39	3.02	0.81
Int	0.25	0.33	0.44	0.12	0.35	0.54	0.31
HF	0.95	1.31	2.23	0.33	0.63	0.98	<0.0001
VLF/LF	1.18	2.75	4.20	1.81	2.94	4.51	0.28
VLF/HF	1.08	2.75	4.34	4.42	6.70	9.61	0.0007
LF/HF	0.55	0.98	1.74	1.57	2.23	3.51	0.004
Total power	4.03	7.44	10.55	3.25	7.44	13.23	0.70

Note: Measures include time domain assessments of rate (IBI = mean interbeat interval; HR = mean heart rate), total variability (SDNN = standard deviation of IBIs; RMSSD = root mean square of differences between IBIs; (MSD = mean of absolute value of consecutive IBI differences; Log HRV = natural log of variance of IBI time series), an estimate of parasympathetically controlled variability Log RSA = natural log of variance of filtered (0.4–1.7 Hz) IBI time series). Frequency domain measures of band-specific power (integrals in bpm^2) include very low frequency (VLF) [0.02–0.08 Hz], low frequency (LF) [0.08–0.2 Hz], intermediate (Int) [0.2–0.4 Hz], high frequency [0.4–1.7 Hz]

Application of Biomagnetometry to Investigate Group Differences

The strength of biomagnetometry lies in the ability to reliably detect fetal MCG in the second and third trimesters and to identify fetal movements that influence HRV and fetal state. Because our research interest is focused on developmental programming we tested whether metrics of fetal HRV were sensitive enough to detect differences in groups where maternal factors might influence the development of cardiac autonomic control. For example, it is known that maternal factors such as over or under-nutrition, maternal obesity, and gestational weight gain or gestational diabetes can influence endocrine status during fetal development thereby altering offspring physiology and metabolism (Godfrey & Barker, 2000). What was not known is whether changes in developmental physiology were detectable in utero. Therefore, we used fetal biomagnetometry to measure group differences in two separate research studies. The first was a pilot study designed to test the effect of maternal aerobic exercise on the longitudinal development of fetal and infant cardiac autonomic control. The second was a randomized clinical trial designed to test the effect of maternal docosahexaenoic acid (DHA) supplementation on fetal cardiac autonomic control and newborn neurobehavior.

The Effect of Maternal Exercise

We aimed to test the effects of maternal exercise during pregnancy on the development of fetal cardiac autonomic control and questioned whether longitudinal measures of HRV during the fetal period and into infancy could serve as a sensitive indicator of programming effects. This was a prospective, non-blinded, longitudinal pilot with maternal–fetal MCGs recorded at 28, 32, and 36 weeks of gestational age. A subset of women was enrolled in a follow-up study when their infants were 1 month of age. Sixty-six women enrolled in the fetal portion of the study (May, Glaros, Yeh, Clapp, & Gustafson, 2010) and 46 participated in the infant follow-up (May, Scholtz, Suminski, & Gustafson, 2014). Women were categorized into Exercise or Control group based on their responses to a physical activity questionnaire validated for use during pregnancy. Women were assigned to the Exercise group if they continued moderate to vigorous aerobic exercise throughout their pregnancy for a minimum of 30 min, three times/week. This criterion was based on minimum recommendation of the American College of Obstetricians and Gynecologists (ACOG) for women previously sedentary prior to pregnancy. ("ACOG Committee opinion. Number 267, January 2002: exercise during pregnancy and the postpartum period," 2002.)

Simultaneous MCG recordings were obtained and data processed as described in the "Methods" section of this chapter. For the infant MCG, the recordings were obtained by placing the infant in a foam seat support system with the chest approximately 10–20 cm from the sensor array. If infants became agitated, women were asked to hold the infant to their chest with the infants' back facing the sensor array.

The time-domain metrics, SDNN and RMSSD were used as measures of overall and short-term HRV respectively. Frequency domain metrics calculated from IBI time-series were restricted to frequency bands reported for the fetus (David et al., 2007). For infant analysis, we used the following frequency bands: LF, 0.04–0.2 Hz; HF, 0.2–1.5 Hz. A retrospective analysis of HRV associated with fetal breathing movements was done in the same cohort (Gustafson, May, Yeh, Million, & Allen, 2012) after characterizing the dMMG associated with periodic fetal breathing movements (Gustafson et al., 2011). All women delivered healthy, term singleton pregnancies. There were no group differences for maternal age, prepregnancy BMI, or education.

Fetal portion. No group differences were observed prior to 36 weeks of gestational age. Forty women kept their 36-week appointment (Control $n=20$, Exercise $n=20$). After adjusting for gestational age and fetal activity state (both were significant factors for all cardiac outcome variables), fetal HR was lower in the Exercise group ($p=0.023$), while the time-domain metric,

RMSSD was higher ($p=0.024$). There was a trend for higher SDNN in the Exercise group ($p=0.06$). The frequency domain metrics, VLF, LF, and HF power were significantly higher in the Exercise group ($p=0.026$, 0.035, and 0.021, respectively). These effects were primarily seen when the fetus was in the active state. Further analysis of the effect of maternal exercise "dose" (intensity and duration) revealed that women who engaged in higher intensity physical activity had fetuses with lower HR and greater overall HRV. The longer the duration of maternal physical activity, the greater the increase in fetal HRV in both time and frequency domains (May, Suminski, Langaker, Yeh, & Gustafson, 2012).

Interaction between fetal breathing movements and maternal exercise. In order to understand how periodic fetal breathing activity influenced fetal HR and HRV, we compared metrics during periods of fetal breathing and apnea (Gustafson et al., 2011). We then performed a retrospective analysis of the data to determine if maternal physical activity moderates the effect of fetal breathing (Gustafson et al., 2012). The analysis was limited to 36 weeks of gestational age, fetal active state, with identifiable fetal breathing epochs lasting 1 min or longer (Control; $n=15$, Exercise; $n=15$). We found significant main effects of maternal exercise and fetal breathing, as described previously. The novel finding in this analysis was the significant interaction between maternal exercise and fetal breathing for measures of overall HRV (Log HRV; $p=0.002$) and the frequency domain metrics, Total Power, VLF, Intermediate, and HF Power ($p=0.006$, 0.01, 0.007, and 0.004, respectively). Maternal exercise effects were only seen during periods of breathing activity, a time when fetal vagal function is enhanced; however, the effect extended to sympathetic function as well (or perhaps less sympathetic withdrawal).

Infant portion: Forty-three women agreed to participate in the study when their infants were age 1 month (Control $n=27$, Exercise $n=16$). Our aim in this arm of the study was to determine whether the observed findings during the fetal period were an in utero phenomenon or whether similar findings would be observed 1 month after birth. The latter would suggest that maternal physical activity has the potential to program fetal cardiac autonomic nervous system in favor of higher HRV. Infants were alert and quiet during the recording in order to minimize differences in state.

Infants born to women in the Exercise group had higher RMSSD ($p=0.01$), LF and HF power ($p=0.002$, 0.004, respectively). Infant HR remained lower in the Exercise group (147 vs. 153 bpm in Control) and SDNN continued to be higher, but neither variable reached significance; HR ($p=0.185$), SDNN ($p=0.342$).

These data are from a relatively small, pilot study that was designed to measure the effect of maternal exercise during pregnancy on fetal cardiac autonomic control. The study had several limitations: (a) it was not blinded, (b) made use of a questionnaire that can be subject to bias, and (c) did not register maternal fitness level before pregnancy. Nonetheless, the twofold aim of the study was achieved and the results can serve in designing future controlled studies.

Earlier studies demonstrated the maternal benefits of staying active during pregnancy with no harm to the fetus, but no study had demonstrated a potential fetal benefit, although it was recognized that the intrauterine environment could influence fetal cardiac autonomic control. (Galland, Taylor, Bolton, & Sayers, 2006). By using maternal–fetal MCG, we were able to show that physical activity during pregnancy can influence the development of fetal cardiac autonomic control, that the duration and intensity of the exercise is a factor, and that the effects endure into the infant period, thereby confirming that this is not an in utero phenomenon, but instead a programming effect.

The Effect of Maternal Docosahexaenoic Acid (DHA) Supplementation

Studies in adults have demonstrated consistently that dietary intake of fish and/or long chain polyunsaturated fatty acid (LCPUFA) supplements

reduce heart rate (HR) and improve measures of HRV, suggesting that LCPUFAs have an effect on cardiac autonomic function (Christensen, 2003; Christensen & Schmidt, 2007; Holguin et al., 2005). Term infants fed milk or soy-based formulas with DHA had lower HR and higher HRV than infants fed similar formulas without DHA (Pivik, Dykman, Jing, Gilchrist, & Badger, 2009). We showed that term infants fed formulas with DHA as 0.32, 0.64, or 0.96 % total fatty acids [with 0.64 % arachidonic acid (ARA), 20:4*n*-6] had lower HR at 4, 6 and 9 months of age than infants in the control group fed formula without DHA and ARA (Colombo et al., 2011). When these infants were administered a visual habituation protocol that yields both behavioral and psychophysical indices of attention, infants who received the DHA at 0.32 and 0.64 % spent more time engaged in active stimulus processing (active phase of attention) than infants fed unsupplemented formula. We also observed that fetal HR was lower and HRV higher in women who self-reported prenatal intake of DHA alone or in combination with EPA (20:5*n*-3) in a pilot study (Gustafson, Colombo, & Carlson, 2008). This observation led us to conduct a clinical trial (Gustafson et al., 2013). Our preliminary results and those from the previously cited studies suggest that prenatal and postnatal supplementation of DHA (alone or in combination with EPA or ARA) may have an effect on fetal and infant cardiac autonomic function, similar to what has been observed in adults. We tested the hypothesis that supplementing pregnant women with 600 mg/day of DHA during the second and third trimesters of pregnancy would result in lower fetal HR and higher HRV.

The clinical trial was a longitudinal, randomized, double-blind, placebo-controlled study conducted at the University of Kansas Medical Center in Kansas City, Kansas, USA. Women were included in the study if they were between 16 and 35.9 years of age and carrying a singleton pregnancy between the 12th and 20th week of gestation. After screening for exclusion criteria, 67 eligible subjects were randomly assigned to the capsule allocation (600 mg DHA or Placebo oil). MCGs were recorded at 24, 32, and 36 weeks of gestational age. All subjects gave written, informed consent prior to enrolling in the study. The study was approved by the Human Subjects Committee at the University of Kansas in accordance with the Helsinki Declaration of 1975 as revised in 1983 and was overseen by a Data Safety and Monitoring Board.

The methods of MCG recording and data processing were consistent with those described earlier in the Chapter. We found a significant effect of advancing gestational age on all metrics of fetal HR and HRV. This was expected given that fetal HR decreases and HRV increases with advancing gestational age. Trends towards lower fetal HR ($p = 0.095$) and longer IBIs ($p = 0.074$) were observed in the DHA supplemented group (effect sizes estimated from the differences in LS means were 0.40 and 0.44, respectively). For the time-domain metrics representing overall HRV (SDNN) and short-term HRV (RMSSD), maternal DHA supplementation resulted in significantly higher values on both metrics of fetal HRV (SDNN; $p = 0.017$, RMSSD; $p = 0.007$). Similarly, the frequency-domain metrics that reflect a combination of sympathetic and parasympathetic input (VLF, LF power) are significantly higher in the supplemented group (VLF; $p = 0.013$, LF; $p = 0.014$). The putative parasympathetic metric, HF power, was higher in the group randomized to DHA intervention, though falling just short of conventional levels of significance ($p = 0.06$). DHA supplementation had no effect on cardiac autonomic balance as reflected by group comparisons of the frequency band ratios. There were no significant group-by-time interactions seen for any metric of fetal HR or HRV.

The results largely supported our hypothesis that DHA supplementation during pregnancy would increase fetal HRV. We find that the effect extends to both branches of the developing ANS as evidenced by greater overall HRV (SDNN) and greater power in frequency bands that contain both sympathetic and parasympathetic input (VLF, LF). Furthermore, the frequency band ratios, thought to represent autonomic balance, were not different between groups. Thus, the effect of DHA on fetal cardiac autonomic control was global, rather than constrained or specific to one arm of the ANS.

The period where we observed the greatest group difference in metrics of fetal HRV occurred in the third trimester. This was expected since variability in fetal HR begins to emerge between 32 and 34 weeks of gestational age largely due to increased vagal inhibition (Groome, Loizou, Holland, Smith, & Hoff, 1999). Another fundamental event in the development of brainstem mediated systems during the third trimester is the emergence of fetal sleep states associated with activity or quiescence (Mirmiran, Maas, & Ariagno, 2003). The maturation of these two endogenously regulated, brain-stem mediated systems coincides with the period in which there is greatest DHA accrual into the developing fetal brain and nervous system (Clandinin et al., 1980).

Given the study's focus on the autonomic outcomes of DHA prenatal supplementation, we also included the Neonatal Behavioral Assessment Scale (NBAS) as an outcome measure. The NBAS yields measures of state, arousal, physiological reactivity and fundamental forms of attention (Brazelton & Nugent, 1995). Findings of improved fetal HRV in the DHA-supplemented group were strengthened by positive findings from the infant NBAS. Two NBAS clusters (motor and autonomic) were significantly higher in infants whose mothers received DHA during pregnancy; higher scores on these clusters reflect improved function in specific neurobehavioral domains. In particular, the autonomic cluster is thought to reflect the quality of the newborn's physiological reactivity to the environment; more mature autonomic function is an indication of flexibility and integrity of the ANS and is ultimately thought to be related to cognitive functions. These findings, coupled with the positive effects on fetal HRV are consistent with the hypothesis that the effect of DHA on development may be mediated through the ANS (Gustafson et al., 2008).

Conclusions and Future Directions

In this chapter, we described methods of fetal biomagnetometry and applied HRV analysis, three biomagnetic field signatures associated with fetal neurobehaviors that influence HRV and state, and the results of two studies designed to utilize fetal biomagnetometry as a research tool to detect developmental programming effects. Fetal NNS, hiccups, and breathing movements result in distinct, biomagnetic signatures that, because of their independent source, are clearly identified using ICA. We show that these neurobehaviors modulate fetal HRV and suggest that this may reflect the developmental integration of coupled oscillators. By using longitudinal measures of fetal HRV, we show that maternal factors such as physical activity and increased dietary intake of DHA during gestation have a positive effect on the development of fetal and infant cardiac autonomic control and newborn neurobehavior.

Typically, birth weight has been used as a proxy for developmental programming effects; however, programming effects can range across the entire spectrum of birth weight, limiting its use as a sensitive indicator. Using birth weight as a proxy does not explain the neural mechanisms behind group differences or observed associations. In order to make recommendations and policy decisions with regard to the health of women and their offspring, we must understand effects on physiology and neural integration. To that end, fetal biomagnetometry shows promise as a noninvasive and sensitive method.

It is also important to consider metrics of HRV as more than a measure of heart function but rather as a proxy for the degree of integration between central and peripheral nervous systems in the developing fetus. Recent investigations have described periods of maternal–fetal synchrony (Van Leeuwen, Geue, Lange, Cysarz et al., 2003) and highlighted the significance of individuality (Van Leeuwen et al., 2013), suggesting the potential for in utero programming of heart rate variability (HRV). The transition from fetal to newborn life requires enormous physiologic adjustments. Autonomic function is critical for reacting to changes in environment and needs to be flexible and variable for proper development. Porges and Furman (2011) propose that the maturation and myelination of the autonomic nervous system during fetal development and the first year of life provides the neural foundation to

adapt to a changing and often challenging environment and that the response needs to be flexible for the development of social engagement and cognitive development. As such, the fetal ANS may be a target for developmental programming effects, making it ideally suited for research investigation.

The human organism might be considered as a network of interactions between integrated physiological systems. Fetal cardiac autonomic control, movements, behaviors, and control of state are all part of a developmental repertoire, linked to the neural integration of oscillating systems that prepare the fetus for life outside the womb. Coordination of this developing network sets the stage for post-natal neurobehaviors including feeding, breathing, sleep, arousal and attention. We are challenged with how to measure the output of the network and this work is further complicated by nonlinear interactions and lack of understanding of coupling mechanisms. Nonetheless, advances in analytical techniques make future work in this area promising.

Noninvasive, longitudinal measures are essential for the continuing investigation of the ontogeny of behavioral development in the human fetus. The ability of biomagnetometry to detect and measure fetal physiologic systems under neural regulation creates an opportunity to identify a network of interactions and then to study the development of the network as it relates to differences in fetal state and behaviors, interactions with the maternal unit and reactions to various stimuli. This work serves as foundation for future studies that will determine how the fetus adapts as an individual to the in utero environment while providing physiologic biomarkers for the evaluation of interventions.

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Afterword

Collectively, the chapters in this volume provide perspectives on future directions for fetal research, particularly in the area of behavior observed through body movement and heart rate changes and environmental perturbations. The diversity of issues discussed indicate a need for a unifying theory of fetal development to answer questions such as when and how brain development is reflected in specific behavioral markers and under what circumstances the brain needs to be stimulated through behavior, highlighting behavior as the crucial factor leading to brain development. Knowledge and understanding of when and how behavior drives central nervous system maturation and when and how central nervous system maturation drives behavior is essential for a thorough conceptualization of development and needs to be investigated throughout gestation in typical and atypical pregnancies. A multidisciplinary approach including animal models and human fetuses incorporating genetic, epigenetic, behavioral, and imaging research techniques will benefit such enquiry.

More specifically, within and across laboratories, researchers raise common questions and propose novel strategies for advancing the field. With regard to the study of fetal movements, multidisciplinary approaches highlight common questions such as how one might reconcile reports of low inter- and intra-fetal consistency. Recent research examining developmental progression indicates that observing fetuses during windows of predefined activity might reduce random variation. In animal models, variation can be reduced by experimentally imposing structure not possible with humans, such as the use of knockout mice to control the function of certain genes or by restricting movements artificially in order to control for their effects in normal maturation. In future, more precise definitions of particular movements as well as increased visibility by better imaging technology could lead to enhanced uses of movements as indicators of fetal health.

Also in future, fetal heart rate signals captured using sophisticated imaging technology requiring the formulation of new algorithms for analyses might be taken into account when defining indices, improving the performance of computer fetal heart rate analysis systems. Similarly, improved study designs and large sample sizes combined with postnatal follow up of prenatally tested fetuses could allow for identification of fetuses at risk for later delays or potentially problematic development. Given the importance of reducing morbidity in human development, most research concentrates on ill health as an end point against which to measure fetal development. However, currently given the wide variation in what might be termed ‘normal’, measures of optimal behavior are missing and could theoretically be used as standards against which to compare results from various laboratories.

Factors influencing fetal development, including maternal emotions and behavior during pregnancy, are being identified based on postnatal outcomes. Thus, researchers in this volume call for future investigations to concen-

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trate more fully on both positive (e.g., expression of appropriate emotions and awareness of the infant's potential and limits) and negative (e.g., stress, anxiety, depression) influences of maternal emotions and positive (e.g., exercise) as well as negative (e.g., alcohol ingestion) maternal behaviors in determining prenatal influence on postnatal outcome. Given current limitations to our understanding of the underlying mechanisms of psychological and physical effects on biological function, such as the role of pro-inflammatory cytokines in pregnancy, more fundamental scientific research is needed before targeted interventions can be designed and tested. There also are limits imposed on current research based on our understanding of complex concepts such as stress or depression especially given that pregnancy presents a unique challenge because of the normal psychological and physiological changes occurring over gestation. Clearly, the field of epigenetics will continue to become more prominent, facilitating innovative interventions to optimize outcome.

Both invasive techniques restricted to animal models as well as noninvasive longitudinal measures are essential for the continuing investigation of the ontogeny of behavioral development in the human fetus using not only sophisticated 4D imaging techniques but also biomagnetometry to detect and measure fetal behavioral and physiological systems under neural regulation. This creates an opportunity to identify interrelationships as well as to study interactions and the developmental trajectories within the maternal–fetal system. Fetal cardiac autonomic control, behaviors, and control of state are all part of a developmental repertoire, linked to the neural integration of oscillating systems that prepare the fetus for life outside the womb. Coordination of this developing network sets the stage for postnatal neurobehaviors including feeding, breathing, sleep, arousal, and attention. There is still a challenge identified with how to measure the output of the network. Nonetheless, advances in analytical techniques make future work in this area promising, enabling advances in methods which could improve the prediction of fetal health not only at the time of observation but also in the longer term.

The issue of whether standardized procedures help or hinder discovery is raised in this book with different points of view expressed. Some researchers argue for the importance of future research to be more standardized by controlling stimuli uniformly, whereas others argue the opposite. Accordingly, some authors suggest that individual variation is essential in order to discover natural development without imposing restrictions which might hide the very development to be studied. Hence, they posit that by adjusting the intensity level or duration of a stimulus for each individual fetus it could be that additional more sensitive measures will provide richer or more precise descriptions of typical and atypical behavior. This may be particularly important in situations when criteria for determining a diagnosis is difficult to establish such as in the case of fetal alcohol exposure.

In summary, contributors to this book suggest that more integrated genetic–epigenetic, brain–behavior, and preclinical–clinical research needs to be conducted. They also propose that more attention should be directed to the maturational continuity across the prenatal–postnatal divide. Importantly, more exclusive definitions and interpretations of normal and abnormal behavior need to be provided. Such efforts by the scientific community directed to studying the prenatal period will ultimately improve prediction of offspring outcome and lead to targeted interventions to facilitate optimal long-term development.

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