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Functional connectivity of the human brain *in utero*

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Abstract

The brain is subject to dramatic developmental processes during the prenatal period. Nevertheless, information about the development of functional brain networks during gestation is scarce. Until recently it has not been possible to probe function in the living human fetal brain. Advances in functional MRI have changed the paradigm, making it possible to measure spontaneous activity in the fetal brain and to cross-correlate functional signals to attain information about neural connectional architecture across human gestation. We summarize the earliest MRI studies of fetal neural functional connectivity and highlight unique challenges and limitations inherent in the technique. In addition, we discuss future directions to unlock the potential of fetal brain functional MRI research.

Keywords

Fetus; Functional Connectivity; Brain Development; Pregnancy; Resting-state

Exploring fetal functional brain connectivity *in utero*

During the prenatal period, the brain undergoes dramatic developmental processes [1]. Already at week 7 of gestation, production of neurons starts at an astonishing pace of 250,000 per minute [2]. At the end of the second trimester, billions of neurons are produced and comparatively few will be produced after this time [3]. Another striking accomplishment of fetal brain growth is the elaboration of neural connections. By the 16th week of gestation, neuronal connections become active as synapses rapidly form [4].

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Given the dramatic developmental change occurring before birth, the prenatal period is both a window of opportunity and a period of great vulnerability to environmental insults [1, 5, 6]. Accumulating research shows that abnormal development of the brain during fetal life contributes to the etiology of many neuropsychological disorders [7]. Moreover, altered brain connectivity has been suggested to be a key feature of several neuropsychiatric disorders [8–10]. Examining network formation and function in this period of extreme plasticity may therefore have the potential to produce unique discoveries that can help us to understand and prevent developmental psychopathology in the future.

Over recent decades, significant insights about fetal structural brain connectivity have been derived from postmortem and animal experiments (for a review, see [11, 12]). Less is known about the emergence and maturation of functional activity or coordination of activity across brain areas before term birth. Knowledge about prenatal development of large-scale, functionally connected brain circuitry has primarily been derived from resting-state functional magnetic resonance imaging (fMRI) studies applied to prematurely born infants (e.g., [13–17]). Although results from postmortem, animal and prematurely born infants provided useful insights into fetal neural network development before term birth, translating the results from these studies to normal fetal brain development remains questionable (see Box 1).

Box 1

Scanning the preterm infant as an alternative: Issues

As an alternative to scanning fetuses *in utero*, researchers have scanned prematurely born infants to elucidate brain functional connectivity (FC) development before the normal time of birth [e.g., 13, 14–16]. Although these studies have provided useful insights into brain FC development in the antenatal period, the use of premature infants as model for intrauterine fetal brain development has shortcomings. Preterm exposure to the extra uterine world in a period of rapid neuronal proliferation and cell differentiation is likely to influence brain development in several ways. First of all, preterm infants are often exposed to hospitalization in the neonatal intensive care unit (NICU). Stressors in the NICU, such as intubation, have been associated with altered functional connectivity in the temporal lobes and abnormalities in motor behavior in preterm infants [56].

Prematurely born infants are also more likely to be exposed to pain, as they are no longer protected by the womb and often have to undergo invasive procedures. Studies that investigated the effect of pain exposure in preterm infants admitted to NICU found higher early life procedural pain was associated with altered brain structure (i.e., reduced white/gray matter and thinner cortex) [57, 58]. In addition, preterm infants are also exposed to different stimuli, such as noise and light exposure in the NICU and a lack of contingent infant-controlled social interaction [59].

Importantly, it may not only be the addition of exposures that modulates brain development in preterm infants, but also the lack of intrauterine exposures in late pregnancy. For example, premature birth produces deprivation from a variety of hormones that are normally provided during (late) pregnancy, such as IGF-1, estrogens, progesterone and thyroid hormones [60, 61]. In addition, premature infants may miss out

on important nutrients, such as cholesterol, Long chain polyunsaturated fatty acids, and vitamin K [60]. Hormonal and nutrient deprivation may play a critical and over-looked role in the etiology of white matter pathology in preterm infants [60]. Taken together, exposure to the extra uterine environment and deprivation from key intrauterine signals in late pregnancy related to preterm birth seems to shape the infant brain differently than exposure to normal, term pregnancy

Recent methodological advancements in functional MRI have provided researchers with an alternative: examining functional connectivity development of the human fetus, *in utero*. With the use of resting-state fMRI researchers are able to map ‘functional connectivity’ (FC) based on inferences drawn from covariance in spontaneous brain activity across spatially discrete brain regions (for a review, see [18, 19]). This approach takes advantage of a fundamental neural property: brain areas that comprise functional brain systems (e.g., the motor system) continue to communicate even when the subject remains perfectly stationary and not engaged in a specific task [20]. By applying resting-state fMRI (rs-fMRI) to the fetal brain, for the first time, it is possible to non-invasively examine fetal functional brain circuits before birth (see Figure 1). Resting-state fMRI, in contrast to task-based fMRI, requires no active participation from the subject, no controlled stimulus presentation, and no comparison of activity levels [21], making it a very suitable technique for studying brain function in young children, including fetuses (see Box 2 for safety considerations). This review summarizes the earliest studies of fetal neural FC, highlights unique challenges and limitations inherent in the technique, and suggests future directions for the field (see Outstanding Questions Box).

Box 2

Safety considerations for the fetus

Several studies have followed up with subjects scanned during the third trimester and reported no harmful effects related to scanning [62, 63]. There are two main concerns when considering fetal safety: heat and sound. Tissue heating can result from radiofrequency energy used during scanning. When a subject is scanned, the radiofrequency pulses of the fMRI sequence interact with magnetic variations of different tissue to make images, and this process involves energy that is passed into the subject’s body. Because the fetus has very limited capacity to efficiently lose heat, extra precautions need to be undertaken when designing fetal MRI research studies. The field strength of the scanner is an important factor to consider, since energy deposition has potential to increase with increasing field strength. Fortunately, it is possible to select scanning parameters that are low in heat energy that are more ideally suited for fetal MRI. For example, decreasing the number of slices, increasing repetition time, and the use of parallel imaging techniques can all decrease the absorptions rate [64]. Another concern of fetal imaging with MRI is a potential detrimental effect of the scanning noise. Nevertheless, no evidence of distress of the fetus during scanning [65] or hearing deficits in children scanned prenatally [63] have been observed. Still, several precautions can be taken into consideration to lower the noise level during the scan, which can be achieved by altering scan parameters or using different sequences.

Criteria for safety can fail when the fetus is not the intended subject of an MRI scanning protocol. Research volunteers are often asked during screening procedures if the individual is intending to become or may be pregnant. This is a circumstance in which the fetus is not the intended target of the study and parameters are not designed with fetal exposure in mind. Given that low energy is almost ubiquitously used in research studies, heating and sound to the fetus, which may be some distance from the maternal anatomy being imaged, are not likely to be of any concern; however, all must advocate for careful screening wherein the intended fetus scanned is considered carefully in design of the imaging protocol. In summary, when safety precautions are taken seriously, scanning the fetus with fMRI can be viewed as a safe procedure for both clinical and research purposes.

Outstanding Questions Box

- Does the fetal brain have functional “hubs”, or highly connected regions important for integration of activity across brain regions? How similar or different are these from regions that operate as hubs in the mature brain, such as the posterior cingulate cortex?
- Can fetal functional connectivity predict child outcomes, such as intelligence and psychopathology?
- Does adversity during pregnancy (e.g., maternal anxiety/depression, nutrient restriction, maternal alcohol exposure) alter the fetal brain? And if so, does altered fetal brain connectivity mediate the association between prenatal exposure to adversity and child outcomes?
- How are fetal structural and functional connectivity development interrelated?
- Once we identify features of the fetal connectome that predict negative long-term outcome, what possibilities exist for modifying network connectivity even before birth?

Development of functional brain connectivity before birth

Very few studies have used rs-fMRI to discover novel properties of neurodevelopment in the human fetus [22–26].

Resting-state studies in full-term [27] and preterm infants [15, 16] have suggested the existence of a “proto”, or partial, default mode network (DMN) in the early newborn. The DMN is an organized, baseline default mode of brain activation that is observed in adults [28] and children [29]. Fetal rs-fMRI examination offers the first opportunity to confirm prenatal origins of this network and to evaluate processes by which it becomes organized prior to birth. Recently, fetal rs-fMRI studies have shown that primitive forms of functional networks appear to be present by mid gestation [21, 25], including a primitive form of the DMN. This indicates that a “proto” DMN may already be present in the fetus. In addition, the posterior cingulate cortex (PCC), an important component of the adult DMN, was

identified as a central “hub” in overall brain connectivity in infants [30], and recently it has been suggested that intrauterine establishment of PCC connectivity may be foundational to the emergent architecture of the developing human brain [21, 23]. The PCC may therefore be an important precursor of the adult DMN, but more research is necessary to confirm this notion.

In addition, fetal rs-fMRI research has explored age-related changes in modularity of the fetal brain [24]. Modularity refers to a pattern of connectivity characterized by groups of highly inter-connected brain regions (so-called “modules”), which are relatively sparsely connected to regions in other modules. High modularity is characterized by higher connectivity within-modules than in between-modules. There are several advantages to modular network organization, including greater robustness, adaptivity, and evolvability of network function [31]. Recent research has found that the human fetal brain also shows modular organization and that modules comprise areas that will later support vision, movement, language, and data integration [24]. In addition, connections within the modules were stronger in older compared to younger fetuses, while the connections between modules were weaker for older fetuses [24]. These results together suggest that in early life the brain is first focused on the development of independent functioning systems (segregation) and only with time will start develop integration as a whole brain system.

Furthermore, research on the fetal brain revealed that fetal functional connectivity development may follow both medial-to-lateral [23, 26] and posterior-to-anterior [25] patterns of development. Evidence for medial-to-lateral connectivity development in the fetal brain can be established by showing that connectivity between midline regions is stronger than connectivity of more lateral regions, while evidence for posterior-to-anterior development can be established by showing that connectivity between posterior areas is stronger than between anterior regions. A medial to lateral development of brain FC has also been demonstrated in preterm infants [16], with connections between cross-hemispheric homologues, or mirror regions, evident as early as 26 weeks of gestation.

In sum, these studies show that primitive forms of functional networks, such as the DMN, appear to be present by mid gestation. Furthermore, long-range, short-range, cross-hemispheric and inter-modular connections increase over the course of gestation [22, 23, 25], while connectivity between modules decreases [23]. In addition, fetal functional connectivity development may follow both a medial-to-lateral [23, 26] and posterior-to-anterior [25] patterns of development. Dramatic changes in functional connectivity development and the emergence of functional networks before birth emphasize the importance of the prenatal period for brain network formation.

Potential of fetal FC research

Understanding and predicting birth outcomes and child psychopathology

The future of fetal rs-fMRI will likely go beyond advancing knowledge of human neurodevelopment to enhancing understanding of, or even predicting, birth outcomes and child psychopathology *in utero*. The significance of the ability to predict child behavioral and emotional problems before birth is enormous, as it opens windows for very early

prevention and intervention. Intervening at such an early age would have huge implications for the burden on families and cost for society. Nevertheless, before such data can be used for clinical use, fetal functional connectivity data needs to be linked to fetal behavior, birth outcomes, and child behavior.

Recent studies have linked fetal behavior and birth outcomes to fetal FC for the first time. One study examined the relation between fetal eye-movements and fetal brain FC and found that fetal eye-movements were associated to simultaneous networks in visual- and frontal areas of the brain [32]. Very recently, the first study associated fetal FC to prematurity [33] – a relatively common negative birth outcome associated with many complications later in life (e.g., [34]). The authors compared fetal FC between fetuses that would subsequently be born preterm and normally developing fetuses and found that neural functional connectivity was diminished in fetuses that would subsequently be born preterm. More research into this area has the potential to produce revolutionary findings that may change the way we think about and treat developmental disabilities.

Studying fetal programming in utero

Another opportunity for fetal FC research is the study of fetal programming *in utero*. Accumulating research has shown that prenatal exposure to adversity, such as maternal stress or teratogens such as lead and alcohol, can lead to psychopathology later in life (for a review, see [5]). Studying this process *in utero* can illuminate the underlying mechanisms of this association, since it has been suggested that the link between early life adversity and later life emotional and behavioral problems is mediated by altered brain development during gestation. Although an increasing number of studies to date have provided evidence for altered brain structure and function in children prenatally exposed to adversity (e.g., [35–40]), no study to date has used MRI to show adversity-related alterations in the fetal brain. The major disadvantage of collecting the most relevant outcome measures (i.e., brain data) after birth is that results are confounded by postnatal influences. Since the same factors could be driving prenatal adversity may influence postnatal adversity, this is an important limitation. Scanning the brain prenatally permits examination of the impact of prenatal exposure on fetal brain development without the confounding influence of postnatal experiences. Ultimately, using fetal brain data as a mediator between prenatal exposure to adversity and child outcomes may provide new insights into the etiology of psychopathology and open windows for early prevention, starting even before birth.

Future directions to unlock the potential of fetal FC research

The need for longitudinal studies

Although the potential of fetal FC research is compelling, it is of utmost importance that we increase our knowledge about the predictive value of FC measures. Without knowledge about the implications of functional connectivity measures for later life, research in this field will fall short of its potential. To date, studies investigating fetal FC are cross-sectional and, although a cross-sectional design can provide information about aggregate group-level development, it does not allow the investigation of individual development. Examination without measurement of later life functioning, such as child cognitive or emotional

outcomes, is insufficient for isolating features that predict future functioning. No studies have yet linked fetal FC measures to later life outcomes. Thus, we are not able to draw conclusions about the relevance of observations about fetal FC to future human health. Longitudinal designs are a key next step for this burgeoning field, and will benefit in particular from comprehensive biological as well as behavioral characterization over time.

Development of fetal templates and atlases

The registration of fetal fMRI images to a predefined template of the brain provides the opportunity to compare brains between subjects or conditions. Because of the relatively big age-range overlap of preterm studies and *in utero* studies, preterm templates could be usable for studying the fetal brain during the last trimester. Recently, advancements are made to facilitate full brain segmentation in preterm born infants [41]. It is important to note, however, that the preterm brain may be different from that of healthy fetuses in several different ways (see Box 1). In addition, preterm MRI would not be useful for scanning fetal brain in the second or early third trimester.

Although challenging, fetal brain FC research would benefit from the construction of fetal templates. The challenge with fetal templates is that due to the rapidly changing nature of the fetal brain, templates are needed for each brain age at the resolution of weeks gestation. Several prominent groups have constructed these templates and made them publicly available (e.g., [42]), but an issue remains as to normalizing a group of fetuses with a wide range of ages to one single-age template.

In contrast to templates, atlases provide labels in brain anatomy that can be useful for processing and interpretation of fetal data. Several groups are presently working on constructing atlases for the use of fetal MRI data [42, 43]. Nevertheless, as is the case with fetal templates, one atlas of the fetal brain may not suffice because the anatomy of the fetal brain changes dramatically over the course of gestation (e.g., [44]). This will either be solved by generation of representative gestational-age specific fetal atlases, or by improved methodology for subject-specific anatomic segmentation in the fetus. The latter is a non-trivial process, but would result in participant specific structural designations that would benefit fetal fMRI data processing and interpretation. Participant specific segmentation would be robust to fetal age differences, but only if we can assume that segmentation works equally well for all fetal ages. Overall, tools for processing fetal fMRI data are a work in progress, and gestational-age specific brain templates and atlases will be an important next step in enhancing our ability to gain a clearer understanding of early prenatal development.

Motion correction

Movement artifacts can be considered the largest unsolved problem in fetal fMRI. Many analytic techniques developed to overcome movement artifacts in adult brain MRI data cannot be applied to fetal data, where aggregated motion is more variable and substantial, and the object of interest is smaller and inconsistently oriented. Motion in the fetus [45] and mother, as well as physiological signals in both, contribute to changes in image signal intensity that are difficult to separate from signals of interest. Further, when movement involves shifting from one location to another, distortions in the imaging field relative to the

object of interest change in relatedness. In the same vein, magnetization in the field is computed relative to other points in time, under an assumption that the object is stationary. Conventional approaches do not correct for these potential interactions, as they are less of an issue when movement is more constrained, but theoretically it should be possible to correct for these.

The development of automated strategies for correcting movement effects in fetal MRI is an active field of research [46, 47], but best practices have yet to be determined. Many groups now opt for analyzing only those data attained during periods of fetal quiescence, as the fetus tends to alternate between movement and relative inactivity that can last for a minute or more. These groups collect more data than needed and then stitch together the remaining low movement data (approximately 50%) to obtain a long (e.g., 6 min) train of low-movement fetal fMRI time series data [23]. It is also recommended practice to ensure results are robust to movement related error or error correction by determining that main variables of interest (e.g., age, risk group) do not relate to degree of movement, amount of motion censoring, or quantity of data. It is important to note that gestational age of the fetus can correlate with movement. Since older fetuses generally have less space to move, they often move less during scanning. Although this issue is almost completely solved by only selecting quiescent periods of data, it is still important to check correlations between gestational age and movement. See Box 3 for more discussion of challenges inherent in fetal rs-fMRI.

Box 3

Challenges specific to fetal fMRI

There are several challenges specific to fetal imaging. First of all, there is no single orientation of the fetal brain for analysis and the fetus can move during scanning (Figure I, panel a). Because of this, additional processing is needed to manually reorient the fetal brain to fit a spatial template. Further, the lack of fetal atlases and processes for automatic segmentation and registration make analyses more time intensive and restrict region specific isolation of effects. Next, the fetal brain (Figure I, panel b – in orange) is much smaller than the adult brain (Figure I, panel b – in grey), which presents several issues. Reduced size makes the proportion of motion relative to the size of the brain larger, and results in each voxel covering more brain space, driving down the *effective* resolution of each measurement in the brain. Then, of course, motion is one of the most significant concerns in fetal MRI research. While motion artifacts are a problem in all brain imaging research, some concerns are specific to fetal brain MRI. For example, the fetus can move out of the imaging space and/or head position changes can interact with field inhomogeneities to introduce potential measurement error. Furthermore, the fetus is encased within an independently moving maternal compartment (Figure I, panel c), adding the complexity of mother's movements and physiological signals. Finally, the physiological basis of blood oxygen level dependent (BOLD) fMRI measures in the human fetal brain is not well understood. Available data show similarity in hemodynamic response measured using MRI in newborn animals [66] and preterm infants [67], and independent work shows that maternal oxygenation, such as maternal hypoxia, does not

affect the BOLD signal of the fetal brain [68]. Overall, considerable work remains both to optimize fetal fMRI methodology, and to form more precise characterization of the underlying neurophysiology.

Figure 1. Challenges related to fetal fMRI

Panel a shows an example of a fetus with extreme movement. Each image shows the same slice at a different time point. Panel b illustrates that the fetal brain is much smaller than the adult brain. Panel c shows the fetal brain enclosed in the maternal compartment (29 weeks of gestational age).

Combining structural and functional fetal brain connectivity

An alternative and complementary method for understanding connectional architecture of the budding fetal brain is to use MRI diffusion tensor imaging (DTI) sequences. DTI methodology allows for non-invasive visualization and measurement of structural connections of the brain [48]. DTI is sensitive to many microstructural features of the brain and has the ability to reveal the developing axonal connectivity of the fetal brain as well as atypical connectivity in structural brain abnormalities [49]. Previous studies examining structural connectivity in the fetus using DTI have used postmortem fetal brains (e.g., [50, 51]). Due to sensitivity of the technique to movement, there are scant available studies of *in vivo* human fetal DTI [52–54]. It has been suggested that functional brain connectivity is dependent on structural connectivity [55] and recent findings on fetal FC development seem to support this. However, this notion has not been directly examined using human fetal MRI data. Functional fMRI and DTI could complement each other in future fetal research by examining how functional and structural connectivity develop in parallel and interacting ways.

Concluding remarks

Recent methodological advancements in functional MRI have made it possible to examine functional connectivity (FC) development of the human fetus, *in utero*. The potential of this technique not only includes a better understanding of birth outcomes and child behavioral problems, but also opportunities to predict these outcomes before birth. It also provides researchers with the opportunity to study fetal programming in its native environment, *in utero*. Although the potential of fetal FC research is exciting, efforts need to be made to link fetal FC measures to later life outcomes and to improve and develop fetal-specific tools and software. Advancements on these fronts would improve fetal FC research, offering the promise of new insights into the developing human brain.

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References

1. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev.* 2003; 27:3–18. [PubMed: 12732219]
2. Nelson, CA. Neural development and lifelong plasticity. In: Keating, DP., editor. *Nature and nurture in early child development*. Cambridge University Press; 2011. p. 45-69.
3. Berk, LE. *Child Development*. 9. Pearson Education (Us); 2012.
4. Sidman RL, Rakic P. Neuronal migration, with special reference to developing human brain: a review. *Brain Res.* 1973; 62:1–35. [PubMed: 4203033]
5. Bock J, et al. Stress In Utero: Prenatal Programming of Brain Plasticity and Cognition. *Biol Psychiatry.* 2015; 78:315–326. [PubMed: 25863359]
6. Entringer S, et al. Prenatal stress, development, health and disease risk: A psychobiological perspective—2015 Curt Richter Award Paper. *Psychoneuroendocrinology.* 2015; 62:366–375. [PubMed: 26372770]
7. Rees S, Inder T. Fetal and neonatal origins of altered brain development. *Early Hum Dev.* 2005; 81:753–761. [PubMed: 16107304]
8. Matthews M, Fair DA. Research Review: Functional brain connectivity and child psychopathology – overview and methodological considerations for investigators new to the field. *J Child Psychol Psychiatry.* 2015; 56:400–414. [PubMed: 25307115]
9. Dennis EL, Thompson PM. Mapping connectivity in the developing brain. *Int J Dev Neurosci.* 2013; 31:525–542. [PubMed: 23722009]
10. Uddin LQ, et al. Typical and atypical development of functional human brain networks: insights from resting-state fMRI. *Front Syst Neurosci.* 2010; 4
11. Kostovi I, Jovanov-Milošević N. The development of cerebral connections during the first 20–45 weeks' gestation. *Semin Fetal Neonatal Med.* 2006; 11:415–422. [PubMed: 16962836]
12. Tau GZ, Peterson BS. Normal Development of Brain Circuits. *Neuropsychopharmacology.* 2010; 35:147–168. [PubMed: 19794405]
13. Ball G, et al. Rich-club organization of the newborn human brain. *Proc Natl Acad Sci.* 2014; 111:7456–7461. [PubMed: 24799693]
14. Damaraju E, et al. Resting-State Functional Connectivity Differences in Premature Children. *Front Syst Neurosci.* 2010; 4:23. [PubMed: 20725534]
15. Doria V, et al. Emergence of resting state networks in the preterm human brain. *Proc Natl Acad Sci.* 2010; 107:20015–20. [PubMed: 21041625]

16. Smyser CD, et al. Longitudinal Analysis of Neural Network Development in Preterm Infants. *Cerebral Cortex*. 2010; 20:2852–2862. [PubMed: 20237243]
17. Cao M, et al. Early Development of Functional Network Segregation Revealed by Connectomic Analysis of the Preterm Human Brain. *Cerebral Cortex*. 2016
18. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: A review on resting-state fMRI functional connectivity. *Europ Neuropsychopharmacol*. 2010; 20:519–534.
19. Rosazza C, Minati L. Resting-state brain networks: literature review and clinical applications. *Neurol Sci*. 2011; 32:773–785. [PubMed: 21667095]
20. Biswal B, et al. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance Imaging*. 1995; 34:537–541.
21. Anderson AL, Thomason ME. Functional plasticity before the cradle: a review of neural functional imaging in the human fetus. *Neurosci Biobehav Rev*. 2013; 37:2220–32. [PubMed: 23542738]
22. Thomason ME, et al. Age-related increases in long-range connectivity in fetal functional neural connectivity networks in utero. *Dev Cogn Neurosci*. 2015; 11:96–104. [PubMed: 25284273]
23. Thomason ME, et al. Cross-hemispheric functional connectivity in the human fetal brain. *Sci Transl Med*. 2013; 5:173ra24–173ra24.
24. Thomason ME, et al. Intrinsic Functional Brain Architecture Derived from Graph Theoretical Analysis in the Human Fetus. *PloS ONE*. 2014; 9:10.
25. Jakab A, et al. Fetal functional imaging portrays heterogeneous development of emerging human brain networks. *Front Hum Neurosci*. 2014; 8:852. [PubMed: 25374531]
26. Schöpf V, et al. Watching the fetal brain at ‘rest’. *Int J Dev Neurosci*. 2012; 30:11–17. [PubMed: 22044604]
27. Fransson P, et al. Resting-state networks in the infant brain. *Proc Natl Acad Sci*. 2007; 104:15531–15536. [PubMed: 17878310]
28. Raichle ME, et al. A default mode of brain function. *Proc Natl Acad Sci*. 2001; 98:676–682. [PubMed: 11209064]
29. Thomason ME, et al. Default-mode function and task-induced deactivation have overlapping brain substrates in children. *NeuroImage*. 2008; 41:1493–1503. [PubMed: 18482851]
30. Gao W, et al. Evidence on the emergence of the brain’s default network from 2-week-old to 2-year-old healthy pediatric subjects. *Proc Natl Acad Sci*. 2009; 106:6790–6795. [PubMed: 19351894]
31. Meunier D, et al. Modular and hierarchically modular organization of brain networks. *Front Neurosci*. 2010; 4:4–11. [PubMed: 20582258]
32. Schöpf V, et al. The relationship between eye movement and vision develops before birth. *Front Hum Neurosci*. 2014; 8:775. [PubMed: 25324764]
33. Thomason ME, et al. Weak functional connectivity in the human fetal brain prior to preterm birth. *NeuroReport*. in press.
34. Heinonen K, et al. Late Preterm Birth and Neurocognitive Performance in Late Adulthood: A Birth Cohort Study. *Pediatrics*. 2015; 135:e818–e825. [PubMed: 25733746]
35. Buss C, et al. High pregnancy anxiety during mid-gestation is associated with decreased gray matter density in 6–9-year-old children. *Psychoneuroendocrinology*. 2010; 35:141–153. [PubMed: 19674845]
36. Buss C, et al. Maternal cortisol over the course of pregnancy and subsequent child amygdala and hippocampus volumes and affective problems. *Proc Natl Acad Sci*. 2012; 109:E1312–E1319. [PubMed: 22529357]
37. van den Heuvel MI, et al. Maternal mindfulness and anxiety during pregnancy affect infants’ neural responses to sounds. *Soc Cogn Affect Neurosci*. 2015; 10:453–60. [PubMed: 24925904]
38. Rifkin-Graboi A, et al. Antenatal Maternal Anxiety Predicts Variations in Neural Structures Implicated in Anxiety Disorders in Newborns. *J Am Acad Child Adolesc Psychiatry*. 2015; 54:313–321.e2. [PubMed: 25791148]
39. Infante MA, et al. Atypical cortical gyrification in adolescents with histories of heavy prenatal alcohol exposure. *Brain Res*. 2015; 1624:446–454. [PubMed: 26275919]

40. Taylor PA, et al. A DTI-based tractography study of effects on brain structure associated with prenatal alcohol exposure in newborns. *Hum Brain Mapp.* 2015; 36:170–186. [PubMed: 25182535]
41. Yu X, et al. Comprehensive Brain MRI Segmentation in High Risk Preterm Newborns. *PLoS ONE.* 2010; 5:e13874. [PubMed: 21079730]
42. Serag A, et al. Construction of a consistent high-definition spatio-temporal atlas of the developing brain using adaptive kernel regression. *NeuroImage.* 2012; 59:2255–2265. [PubMed: 21985910]
43. Habas PA, et al. A spatiotemporal atlas of MR intensity, tissue probability and shape of the fetal brain with application to segmentation. *NeuroImage.* 2010; 53:460–470. [PubMed: 20600970]
44. Girard N, et al. In vivo MR study of brain maturation in normal fetuses. *Am J Neuroradiol.* 1995; 16:407–413. [PubMed: 7726092]
45. Schöpf V, et al. Functional imaging in the fetus. *Top Mag Reson Imaging.* 2011; 22:113–118.
46. Seshamani S, et al. A Method for Handling Intensity Inhomogeneities in fMRI Sequences of Moving Anatomy of the Early Developing Brain. *Med Image Anal.* 2014; 18:285–300. [PubMed: 24317121]
47. Ferrazzi G, et al. Resting State fMRI in the moving fetus: A robust framework for motion, bias field and spin history correction. *NeuroImage.* 2014; 101:555–568. [PubMed: 25008959]
48. Thomason ME, Thompson PM. Diffusion imaging, white matter, and psychopathology. *Annu Rev Clin Psychol.* 2011; 7:63–85. [PubMed: 21219189]
49. Mukherjee P, McKinstry RC. Diffusion Tensor Imaging and Tractography of Human Brain Development. *Neuroimaging Clinics.* 2006; 16:19–43. [PubMed: 16543084]
50. Huang H, et al. Anatomical Characterization of Human Fetal Brain Development with Diffusion Tensor Magnetic Resonance Imaging. *J Neurosci.* 2009; 29:4263–4273. [PubMed: 19339620]
51. Huang H, et al. White and gray matter development in human fetal, newborn and pediatric brains. *NeuroImage.* 2006; 33:27–38. [PubMed: 16905335]
52. Jakab A, et al. Disrupted developmental organization of the structural connectome in fetuses with corpus callosum agenesis. *NeuroImage.* 2015; 111:277–288. [PubMed: 25725467]
53. Zanin E, et al. White matter maturation of normal human fetal brain. An in vivo diffusion tensor tractography study. *Brain Behav.* 2011; 1:95–108. [PubMed: 22399089]
54. Mitter C, et al. In Vivo Tractography of Fetal Association Fibers. *PLoS ONE.* 2015; 10:e0119536. [PubMed: 25742520]
55. Damoiseaux JS, Greicius MD. Greater than the sum of its parts: a review of studies combining structural connectivity and resting-state functional connectivity. *Brain Struct Funct.* 2009; 213:525–533. [PubMed: 19565262]
56. Smith GC, et al. Neonatal intensive care unit stress is associated with brain development in preterm infants. *Ann Neurol.* 2011; 70:541–549. [PubMed: 21976396]
57. Brummelte S, et al. Procedural pain and brain development in premature newborns. *Ann Neurol.* 2012; 71:385–396. [PubMed: 22374882]
58. Ranger M, et al. Neonatal Pain-Related Stress Predicts Cortical Thickness at Age 7 Years in Children Born Very Preterm. *PLoS ONE.* 2013; 8:e76702. [PubMed: 24204657]
59. Wolke D. Environmental and developmental neonatology. *J Reprod Infant Psychol.* 1987; 5:17–42.
60. Elitt CM, Rosenberg PA. The challenge of understanding cerebral white matter injury in the premature infant. *Neuroscience.* 2014; 276:216–238. [PubMed: 24838063]
61. Berger R, Soder S. Neuroprotection in preterm infants. *BioMed Res Int.* 2015; 2015:257139. [PubMed: 25650134]
62. Kok RD, et al. Absence of harmful effects of magnetic resonance exposure at 1.5 T in utero during the third trimester of pregnancy: a follow-up study. *Magnetic Resonance Imaging.* 2004; 22:851–854. [PubMed: 15234454]
63. Baker PN, et al. A three-year follow-up of children imaged in utero with echo-planar magnetic resonance. *Am J Obstet Gynecol.* 1994; 170:32–33. [PubMed: 8296840]
64. Victoria T, et al. Fetal magnetic resonance imaging: jumping from 1.5 to 3 tesla (preliminary experience). *Pediatr Radiol.* 2014; 44:376–386. [PubMed: 24671739]

65. Vadeyar SH, et al. Effect of fetal magnetic resonance imaging on fetal heart rate patterns. *Am J Obstet Gynecol.* 2000; 182:666–669. [PubMed: 10739527]
66. Colonnese MT, et al. Development of hemodynamic responses and functional connectivity in rat somatosensory cortex. *Nat Neurosci.* 2008; 11:72–79. [PubMed: 18037883]
67. Arichi T, et al. Development of BOLD signal hemodynamic responses in the human brain. *NeuroImage.* 2012; 63:663–673. [PubMed: 22776460]
68. Sørensen A, et al. Changes in human fetal oxygenation during maternal hyperoxia as estimated by BOLD MRI. *Perinat Diagn.* 2013; 33:141–145.
69. Derntl B, et al. Stress matters! Psychophysiological and emotional loadings of pregnant women undergoing fetal magnetic resonance imaging. *BMC Pregnancy Childbirth.* 2015; 15:1–10. [PubMed: 25591791]
70. Brändle J, et al. Heart rate variability parameters and fetal movement complement fetal behavioral states detection via magnetography to monitor neurovegetative development. *Front Hum Neurosci.* 2015; 9:147. [PubMed: 25904855]
71. Monk C, et al. Effects of maternal breathing rate, psychiatric status, and cortisol on fetal heart rate. *Dev Psychobiol.* 2011; 53:221–233. [PubMed: 21400485]
72. Leithner K, et al. Psychological reactions in women undergoing fetal magnetic resonance imaging. *Obstet Gynecol.* 2008; 111:396–402. [PubMed: 18238978]

Box 4**Psychological stress during scanning**

Testing protocols can be tailored to best accommodate the unique needs of pregnant mothers and fetuses. For example, researchers can reduce physical stress by providing additional padding for pregnant mothers, offering them opportunity to choose the position most comfortable to them, and allowing them to take breaks or readjust position between scans. Keeping scan time short also helps in this regard. Psychological comfort can be bolstered by taking any time necessary to explain the procedures and non-invasive nature of the technique, answering any concerns the mother may have about safety, offering to accompany mothers in the room during scanning [69], and offering music or other entertainment during the examination. These precautions are advisable, given that clinically recruited pregnant women report increased anxiety symptoms before scanning, with about one third of the women undergoing fetal MRI reporting it to be “unpleasant” (33.9%) and a few women even recalling it as “hardly bearable” (4.8%) [69, 72]. An important note here is that women included in these studies underwent fetal MRI because of suspected anomalies in the fetus. Volunteer participants are likely to have less stress overall, and frequently report positive experiences associated with scanning, such as seeing pictures of their unborn child and potential to help others. It is also possible to monitor heart rate in both mother and fetus, which can be useful for detecting signs of physiological distress, should they arise [70, 71].

Trends Box

- Recent methodological advancements in functional MRI have made it possible to examine functional connectivity development of the human fetus, *in utero*.
- Fetal resting-state functional connectivity MRI has the potential to advance knowledge of human neurodevelopment to enhance understanding of, or even predict, birth outcomes and child psychopathology *in utero*.
- Only very recently, studies have started to link fetal functional connectivity to fetal behavior and birth outcomes. Longitudinal designs that associate fetal functional connectivity to later life outcomes are a key next step for this burgeoning field.
- The development of age-specific fetal templates and atlases and automated strategies for correcting movement effects in fetal MRI is an active field of research and will likely push the field forward in the future.

Figure 1. Collecting fMRI connectivity data in the human fetal brain in utero

Panel (a) depicts a pregnant research participant placed in a semi-supine position on the MRI table, positioned on their preferred resting side of the body, and given additional padding for comfort. Pregnant women can also be placed on their back (i.e., supine position) for fetal imaging. Here, a wide bore magnet (70cm) provides additional space and a flexible abdominal body coil is wrapped around the waste, secured by a velcro pad that provides further cushion and sound insulation. Panel (b) depicts results from several fetuses scanned in utero ($N = 11$; gestational age = 30–38 weeks) drawn from ongoing research in the lab of Dr. Moriah E. Thomason at Wayne State University. One sample t-tests were used to extract group connectivity averages from seed regions placed (right to left) in the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), and motor hand area. Results are displayed on the brain of 32 and 36 weeks old fetal subjects, for anatomical reference.