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Gaining Insight of Fetal Brain Development with Diffusion MRI and Histology

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Abstract

Human brain is extraordinarily complex and yet its origin is a simple tubular structure. Its development during the fetal period is characterized by a series of accurately organized events which underlie the mechanisms of dramatic structural changes during fetal development. Revealing detailed anatomy at different stages of human fetal brain development provides insight on understanding not only this highly ordered process, but also the neurobiological foundations of cognitive brain disorders such as mental retardation, autism, schizophrenia, bipolar and language impairment. Diffusion tensor imaging (DTI) and histology are complementary tools which are capable of delineating the fetal brain structures at both macroscopic and microscopic level. In this review, the structural development of the fetal brains has been characterized with DTI and histology. Major components of the fetal brain, including cortical plate, fetal white matter and cerebral wall layer between the ventricle and subplate, have been delineated with DTI and histology. Anisotropic metrics derived from DTI were used to quantify the microstructural changes during the dynamic process of human fetal cortical development and prenatal development of other animal models. Fetal white matter pathways have been traced with DTI-based tractography to reveal growth patterns of individual white matter tracts and corticocortical connectivity. These detailed anatomical accounts of the structural changes during fetal period may provide the clues of detecting developmental and cognitive brain disorders at their early stages. The anatomical information from DTI and histology may also provide reference standards for diagnostic radiology of premature newborns.

Keywords

fetal brain; development; DTI; histology; white matter; cerebral wall; connectivity; tractography

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1. Introduction

Delineating the structural changes of human fetal brain is essential to understanding the complicated yet highly organized process of brain development. As complex as the human brain is, its origin is a simple tubular structure. Fig. 1 shows the striking morphological changes of the human brain from the 4th wg (weeks of gestation) to the 40th wg during fetal development. Characterizing the dramatic structural changes of human fetal brain development is not a young field. It has almost two centuries of history. Back to 1816, there was one of the earliest specific accounts of the prenatal human brain (Tiedemann, 1816), followed by reports based on serial sections by known neuroanatomists (e.g. His, 1904; Hochstetter, 1909; Poliakov, 1949; Chi et al., 1977). Comprehensive atlases based on histological slides became available a few years ago (Bayer and Altman, 2004, 2005; O'Rahilly and Moller, 2006). Histology has been a dominant modality and remains to be an important method to study the detailed neural structures of developing brains (e.g. Rakic, 1972; Sidman and Rakic, 1973; Sidman and Rakic, 1982; Rakic, 1988; Honig et al., 1996; Volpe, 2001). A large amount of information can be obtained from histological sections of the fetal brains. Recently developed high resolution diffusion tensor imaging (DTI) technique is complementary to the histology as it is noninvasive, three-dimensional and requires much less imaging time to characterize the entire brain anatomy with the modern scanners. Moreover, its digital character makes it easily distributed and adopted as structural references for both basic neuroscientific studies and clinical diagnoses. However, the best resolution achieved by DTI (*ex vivo* ~100 μ m; *in vivo* ~1500 μ m) can hardly match that of histology (~ μ m). As two complementary techniques, DTI and histology together can reveal both the macrostructural and microstructural anatomical information of developing brains during the fetal period.

Anatomical information revealed by DTI and histology is important for developmental studies in basic neuroscience. The structural atlas of human fetal brain in its two- and three-dimensional (2D and 3D) format will facilitate understanding of the morphology of developmental neural structures. There are many transient structures, such as the ganglionic eminence and subplate, which are unique within specific developmental stages and disappear at term or during the early postnatal development. Moreover, some transient developmental structures, such as the corpus gangliothalamicum, the perireticular nucleus, the intracallosal and subcallosal neurons, transient patterns of the human striatum and the amygdala and nucleus subputaminalis (for review see Judas, 2011), are specific only to human. These unique neural structures found in both the fetal cerebral wall and subcortical nuclei are usually associated with specific neurodevelopmental processes. There are also some neural structures, such as the superior longitudinal fasciculus, that exist in the adult human brain but are completely absent in the fetal brain (Huang et al., 2006; Huang et al., 2009). It is possible for DTI and histology to delineate many of these unique neural structures and provide detailed and rich anatomical information of fetal brains. These brain images from DTI and histology can provide insights into the macrostructural and microstructural patterns of human brain development.

Fetal brain DTI and histology also have the potential to be used for anatomical references for clinical indications in pre- or full-term neonate brains. Routinely used diagnostic methods, such as ultrasound, often have poor sensitivity to significant abnormalities in neonate brains. It has been demonstrated that various injuries, due to perinatal risks, often lead to damage in selective white matter. On the other hand, because of the advances in the critical care of pre-term infants, the survival rate of premature infants has increased dramatically in recent years. Advanced noninvasive DTI technology has been applied directly to the patients to delineate anatomical abnormalities. Precise delineation of the status of specific white matter tracts in the early phase of injuries and the understanding of

injury development may provide more accurate diagnosis and become more important than ever (e.g. Inder et al., 1999; Woodward et al., 2006). The anatomical information from high resolution fetal brain DTI or histology can potentially serve as a clinical reference in diagnostic radiology.

2. Fetal brain DTI and complementary histology

Generally, DTI has been used to characterize the fetal brain anatomy with three different ways, *in vivo* DTI of preterm neonates (e.g. McKinstry et al., 2002; Maas et al., 2004; deLpolyi et al., 2005), *ex vivo* DTI of fixed brain tissues (e.g. Huang et al., 2006, 2009, 2012; Huang, 2010) and *in utero* DTI (e.g. Kasprian et al., 2008). These three fetal brain DTI methods share the same major procedures. The fetal brain DTI procedures from the data acquisition to obtaining DTI-derived maps and conducting DTI-based tractography are briefly outlined in Figure 2.

This review is mainly focused on fetal brain DTI. Since complementary histology is an important technique to cross-validate the DTI findings, we briefly introduced several related histological techniques in section 2.4 below.

2.1 DTI data acquisition and diffusion tensor fitting

DTI (Basser et al., 1994) is based on diffusion magnetic resonance imaging. MRI measures signals from ^1H (proton) nuclei which are magnetic spins in the magnetic field. In DTI studies, we can assume the signals are dominated by water protons. Diffusion magnetic resonance imaging measures water diffusion noninvasively by using the phase difference to detect water motion. Modern MR scanners are usually equipped with three orthogonal gradient systems in the X, Y and Z direction. These gradients have different applications. Depending on these applications, the same gradient systems are named differently. One major application is sampling the spatial space to form MR images thus the gradients are called “imaging gradients” for this purpose. They can also be used to measure diffusion. This function of the gradient systems is emphasized in Fig.2a. A typical diffusion sequence (Stejskal and Tanner, 1965) is featured with a pair of diffusion gradients placed on either side of the refocusing pulse, shown as Fig. 2a. Note that the imaging gradients are also needed but not shown for simplification of the diagram. The frequency of the water proton spin (ω) and the magnetic field B_0 have a simple relationship: $\omega = \gamma B_0$. By adding the gradient, the equation is changed to $\omega = \gamma(B_0 + \mathbf{G}(\mathbf{x}) \cdot \mathbf{x})$ where $\mathbf{G}(\mathbf{x})$ is the gradient strength and $\mathbf{x}$ is the spatial location. After the first gradient, spins at different locations $\mathbf{x}$ have different frequencies and go out of phase as they “see” the different magnetic field strength ($B_0 + \mathbf{G}(\mathbf{x}) \cdot \mathbf{x}$). With the second gradient, only the spin that does not move between two gradient lobes has perfect refocusing. Refocusing results in a strong signal which is bright in the acquired diffusion weighted images (DWI) (Fig. 2a). By combining the X, Y and Z gradients, we can apply the gradient along arbitrary directions. The blue arrows in Fig. 2a indicate the gradient directions. For an organized structure like the human brain, water protons tend to move along the axons rather than perpendicular to them. When the diffusion gradient direction aligns with a specific axonal direction, the signal loss is displayed as dark intensities in the images. For example, the first gradient applies along the horizontal direction (X direction) which is parallel to the axonal directions of the corpus callosum around midline of this axial brain image. Thus the corpus callosum area around the midline is dark in the correspondent diffusion weighted image. The amount of signal loss for those spins with movement is dependent on several parameters, the gradient strength G , the interval of the two gradients Δ and gradient duration δ . This can be described with the following equation:

$$\ln(S/S_0) = -\gamma^2 G^2 \delta^2 (\Delta - \delta/3) D \quad (1)$$

where D is diffusion coefficient, S and S_0 are the diffusion sensitized signal and non-diffusion signal. The complicated term $\gamma^2 G^2 \delta^2 (\Delta - \delta/3)$ can be simplified as a scalar b . Thus equation (1) can be simplified as $\ln(S/S_0) = -bD$.

As diffusion gradients can be applied to arbitrary directions, the diffusion profile composed of diffusion coefficients along these directions can be calculated. For simplification, the diffusion tensor is widely used to characterize the diffusion profile. Fig. 2b shows the tensor fitting process. Since a diffusion tensor has six degrees of freedom, at least six diffusion sensitized images need to be acquired to fit the tensor besides a non-diffusion image. The properties of the three-dimensional ellipsoid are usually defined by the following parameters, the length of the longest, middle and shortest axes (called eigenvalues λ_1 , λ_2 and λ_3) and their orientations (called eigenvectors $\mathbf{v}_1$, $\mathbf{v}_2$ and $\mathbf{v}_3$), shown in Fig. 2b. As the three axes are orthogonal to each other, only six parameters, i.e. λ_1 , λ_2 , λ_3 and x , y , z component of $\mathbf{v}_1$, are independent.

2.2 DTI-derived maps for quantification and contrast

After tensor fitting, DTI-derived images can be obtained from the eigenvalues and eigenvectors of the diffusion tensor. Three DTI-derived images are widely used and shown in Fig. 2c, namely anisotropy map (Beaulieu and Allen, 1994; Chenevert et al., 1990; Hsu and Mori, 1995; Moseley et al., 1990; Turner et al., 1990), color-coded orientation map (or colormap for simplification) (Makris et al., 1997; Pajevic and Pierpaoli, 1999) and apparent diffusion coefficient (ADC) map. These DTI-derived metrics are calculated as follows.

Fractional anisotropy (FA) and relative anisotropy (RA) (Pierpaoli and Basser, 1996) have been widely used to characterize the anisotropy of the tensor. The equations of FA and RA are as follows:

$$FA = \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{2} \sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad (2)$$

$$RA = \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}}{\sqrt{(\lambda_1 + \lambda_2 + \lambda_3)^2}} \quad (3)$$

DTI-derived FA map, ADC map, color-encoded map are shown in Fig. 2c. The values of FA range from 0 to 1. The FA map characterizes the shapes of the diffusion tensor. The higher the value of FA is, the more elongated the shape of diffusion ellipsoid looks like. The eigenvector associated with the largest eigenvalue ($\mathbf{v}_1$) can be used as an indicator of fiber orientation. DTI color-encoded map combines the information of FA and $\mathbf{v}_1$. In the colormap, red (R), green (G), and blue (B) colors are assigned to left-right, anterior-posterior, and superior-inferior orientations, respectively. For the color presentation, 24-bit color is used, in which each RGB color has 8-bit (0–255) intensity levels. The unit vector $\mathbf{v}_1$ ($=v_{1x}, v_{1y}, v_{1z}$) always fulfills the condition: $v_{1x}^2 + v_{1y}^2 + v_{1z}^2 = 1$. Intensity values of $v_{1x}^2 * 255$, $v_{1y}^2 * 255$ and $v_{1z}^2 * 255$ will be assigned to the R(ed), G(reen), and B(lue) channel, respectively. In order to suppress orientation information in isotropic brain regions, the 24-bit color value will be multiplied by FA. The map of apparent diffusion coefficient (ADC) depicts the size of diffusion ellipsoid. ADC is a physical value and usually has the unit of $10^{-3} \text{mm}^2/\text{s}$. It can be obtained with the simple average of the three eigenvalues:

$$ADC = (\lambda_1 + \lambda_2 + \lambda_3) / 3 \quad (4)$$

Detailed review of DTI and its derived metrics can be found in the literature (Basser and Jones, 2002; Beaulieu, 2002; Mori and Zhang, 2006).

2.3 DTI-based tractography

Three-dimensional axonal bundles can be reconstructed from DTI data. The direction of the primary eigenvector ($\mathbf{v}_1$) of the tensor is believed to align with the orientation of its underlying organized structures. DTI-based tractography refers to the techniques of connecting these primary eigenvectors to reconstruct the pathways of the white matter tracts. Streamline propagation methods (Mori et al., 1999; Conturo et al., 1999; Jones et al., 1999; Basser et al., 2000; Poupon et al., 2000; Parker et al., 2002; Lazar et al., 2003) are mostly used. DTI-based tractography requires a fractional anisotropy value higher than threshold and orientation continuity of the primary eigenvector. Left panel of Fig. 2d shows the diagram of a widely used streamline tractography, fiber assignment by continuous tracking (FACT) (Mori et al., 1999). Fiber trackings are initiated from voxel #1 and #2, respectively. With the restricting second region of interest (ROI) which is voxel #3 or #4, two lines are traced. An example of the three-dimensional reconstructed cingulum bundle (red fibers) in the 32 wg fetal brain and traced with this FACT algorithm is shown in the right panel of Fig. 2d. The FACT algorithm with high angular resolution diffusion imaging (HARDI) has also been applied to reveal the fetal brain connectivity (Takahashi et al., 2012) which has been described in details in 4.2 below. Due to the fact that the diffusion tensor model oversimplifies complex neural structures inside the brain, many other tractography algorithms which adapt more sophisticated diffusion models rather than tensor have been postulated. For major white matter tracts, comparison of these DTI-based tractography and postmortem histological slides has shown that tractography based on DTI can reconstruct them accurately (Stieltjes et al., 2001; Catani et al. 2002).

2.4 Complementary histological techniques to study fetal brain development

Before histological sectioning, postmortem fetal brains are usually well fixed with fixative solutions of 4% paraformaldehyde (PFA) or 10% formalin in phosphate-buffered saline (PBS). For example, histological sections immunohistochemically labeled with anti-glial fibrillary acidic protein (GFAP) antibody and neurofilament (NF) antibody are shown in Fig. 4. The process of preparing these histological slides is described as follows. After fixation, the fetal brain tissues were then immersed in 10–30% sucrose in 4% PFA for 2–3 days until they sank in the solution, then frozen in liquid 2-methylbutane at -20°C . Frozen brains were serially sectioned at $80\mu\text{m}$ on a freezing microtome. Sections were stored in a cryoprotectant solution and then transferred into PBS just prior to immunohistochemistry. The immunohistochemistry was performed on floating sections. Sections of postmortem brains were rinsed in PBS before they were blocked in 2% normal goat serum. After they were immunohistochemically labeled with GFAP antibody and NF antibody, the sections were mounted onto gelatinized glass slides, dehydrated through increasing concentrations of ethanol, immersed in two changes of Histo-Clear solution, and coverslipped with DPX mounting medium. GFAP is the main constituent of intermediate filaments in astrocytes and GFAP stain can be used to reveal the architecture of the astrocytes in developing fetal brains. Neurofilaments are a type of intermediate filament that serves as major elements of the cytoskeleton supporting the axon cytoplasm. They are the most abundant fibrillar components of the axon and neurofilament stains can be used to reveal the axons of the fetal brains.

3. DTI of the cerebral wall of human fetal brain

3.1 The microstructural profile of the cortical plate of human fetal brain

As a prominent component of the fetal brain, the cerebral wall (pallium) is the place where extremely complicated yet highly organized development processes occur during fetal development to form the adult cerebral cortex. These developmental processes include proliferation, cell differentiation, synapse formation, axonal and dendritic growth, molecular specification, neural aggregation and myelination. The fetal cerebral wall is characterized by a laminar organization (Kostovi et al., 2002; Kostovi and Vasung, 2009) with some transient layers lacking their direct counterparts in the adult brain. These transient fetal laminae, namely the marginal zone, cortical plate, subplate, intermediate zone, subventricular zone and ventricular zone, have been extensively described (Kostovic and Rakic 1990; Kostovi et al., 2002; Kostovi and Vasung, 2009) and differ in thickness and volume during different stages of prenatal development. These layered structures have also been observed with DTI (Maas et al., 2004; deIpolyi et al., 2005; Huang et al., 2006; Huang et al., 2012) in fetal and preterm brains. From this initial phase of expansion and differentiation, the anatomical (e.g. Toga et al. 2006) and cytoarchitectonic organization (e.g. Economo and Koskinas 1925) of the adult cerebrum emerges. Unique spatio-temporal signatures of the developmental processes in different regions of the cortical plate and subplate (Mrzljak et al., 1988; Kostovic and Rakic 1990; Rakic et al. 1991; Vasung et al. 2010), the two outmost layers of the cerebral wall (except the marginal zone), are visible with magnetic resonance imaging (MRI), and underlie the regional formation of distinct areas. The subplate (Kostovic and Rakic, 1990; Kostovic et al., 2002) is a transient layer in the fetal cerebral wall and almost disappears at term.

A human fetal brain DTI database that delineated the detailed anatomy of neural structures of fetal gray and white matter was established using high-resolution and high-contrast images derived from DTI (Huang et al., 2009). The upper row of Fig. 3 shows the FA map from 13 to 21 wg. The three layers of the cerebral wall could be differentiated at each of the time points during the fetal phase from the FA maps. Qualitative and general trends of the microstructural properties of these layers can be observed. The FA values of the cortical plate were higher than those of the subplate. It was also observed that, in the FA map, the cortical plate becomes darker from the early to late fetal phase of development, indicating loss of anisotropy in the cortical plate. Although a dramatic decrease of FA values in the cortical plate occurs during the development, there is little change in the thickness of this layer. The situation is opposite for the subplate, which is a key transient structure in the middle of the cerebral wall during fetal development. The thickness of the subplate increases dramatically during the fetal phase while this layer remains dark in the FA map and has consistently lower FA values. The FA of the inner layer is higher than that of subplate. The middle row of Fig. 3 shows the characteristic spatio-temporal FA variation of the cortical plate by mapping FA of this layer for fetal brains of 13, 15, 17, 19 and 21 wg. From 13wg to 21 wg, the FA of the overall cortical plate underwent a dramatic decrease, with the highest value of more than 0.5, dropping to around 0.25 to 0.3 in most cortical areas at 21wg (middle row of Fig. 3). The FA decreases are heterogeneous among different cortical regions. For example, the FA decreases at three ROIs, VFC (ventrolateral prefrontal cortex), IPC (posterior inferior parietal cortex) and S1C (somatosensory cortex), demonstrate different patterns (lower row of Fig. 3). Specifically, FA values at IPC are almost flat and FA decrease at VFC is most dramatic among the three while the FA decrease at S1C follows the averaged FA decrease line. Both the decline in the FA of the cortical plate and the constantly low FA values of the subplate within this phase of the fetal brain development seem to reflect certain neurodevelopmental events. The disappearance of densely packed neuronal pattern within the cortical plate due to the development of neuronal dendrites (Mrzljak et al., 1988; Mrzljak et al., 1992; McKinstry et al., 2002; Huang et al., 2012) and

ingrowth/outgrowth of axonal fibers (Kostovic and Judas, 2002; Kostovic and Vasung, 2009; Vasung et al., 2010) could cause the gradual decrease in directional movement of water molecules and result in the gradual decline in anisotropy values (discussed in details in 3.3). In contrast, the subplate zone is rich with water and non-directional (isotropic) movement of water molecules within the subplate zone causes persistently low FA values. The decrease in FA also varies across the cortical plate suggesting regional differences in the maturing pattern. In summary, the FA decline in the future high association areas happens last (lower panel of Figure 3) suggesting the early maturation of the primary ones (central regions) and protracted maturation of future association ones (frontal, parietal and temporal associational cortices).

3.2 DTI and histology for the cerebral wall layer between the subplate and ventricle

The cerebral wall layer between the subplate and ventricle consists of multiple zones (intermediate, subventricular and ventricular zone), which cannot be delineated with the FA map. We call the cerebral wall layer between the subplate and ventricle “inner layer” for simplification (Huang et al., 2012), as it is anatomically located at the most inner part of the cerebral wall, next to the ventricle. Histology could reveal both tangential and radial microstructures in this layer. The FA values in this layer reflect integrated effects of the microstructures within these zones. It can be segmented into two compartments, “close-to-ventricle” (ventricular and subventricular zone) part of inner layer and “close-to-subplate” (intermediate zone) part of inner layer, with the guidance of histological images. The microstructural differences of these two compartments can be quantified with FA. GFAP staining in Fig. 4a and neurofilament staining in Fig. 4e revealed both radial (Fig. 4a) and tangential (Fig. 4e) microstructures, respectively. Microstructures in both directions are indicated by the yellow lines in this layer (Fig. 4b and 4f). The radial microstructures close to the ventricle and most prominent in the areas are likely to be in the ventricular, periventricular and subventricular zones (Fig. 4b). The tangential structures in Fig. 4f delineate the fetal white matter and appear only in part of the inner layer, which is likely to be in the intermediate zone. Darker GFAP staining at the outer edge of inner layer is indicated by the green arrows and may correspond to radial glial end-feet. Combined with the observation from Fig. 4f, showing neurofilament staining of the tangential axonal fibers, it is possible that there are perpendicular crossings of radial and tangential fibers in this part of the inner layer. FA maps of Fig. 4c–4d and Fig. 4g–4h correspond with the histological images of Fig. 4a–b and Fig. 4e–4f, respectively. Due to the crossing of tangential and radial fibers in the inner layer, the FA values at the ROI (Fig. 4c and 4d) with predominantly radial structures are significantly higher (Fig. 4i) than those at the ROI (Fig. 4g and 4h) with mixed tangential and radial fibers.

3.3 Disruption of radial glial scaffold during fetal brain development

During the fetal period, the cerebral wall of the brain displays the structures of both laminated layers and columns radial to the cortical surface. The laminated organization of the cerebral wall has been described in detail in 3.1 and 3.2. During brain development at the embryonic and early fetal phase, there is an active neuronal migration following the radial glia that stretch from the ventricular to the pial surface (Rakic, 1988; Sidman and Rakic 1973). This radial glia structures make the water motion more likely along the radial direction in the cerebral layer. By the 20wg, majority of neurons have reached their final destination in the cortical plate (Bystron et al., 2008). Cortical plate after then is composed of the densely packed post-migratory neurons displaying the radial organization (Kostovic et al., 2002). This property of the cortical plate can be easily seen on the DTI images in *in vivo* and *ex vivo* brains. By visualizing the primary eigenvector of the diffusion tensor of *in vivo* brains of human infants at gestational ages of 26 and 35 weeks, McKinstry and colleagues (McKinstry et al., 2002) found that the radial organization is pronounced at gestational age

of 26 weeks, but disappears by gestational age of 35 weeks, which is shown in Fig. 5. Radial organization is dominant in the cortical plate of the 26 week brain and this radial structure is less evident in the cortex of the 35 week brain (Fig. 5a). Figs. 5b and 5c are the diagrams depicting the relationship between the neural structures in the cerebral layer and the resultant diffusion tensor measurement. The disappearance of the radial organization of the post-migratory neurons, together with the loss of FA, can be caused by dendritic branching of the cortical neurons (Mrzljak et al., 1988; Mrzljak et al., 1992; McKinstry et al., 2002; Huang et al., 2012) and ingrowth/outgrowth of cortical fibers (Kostovic and Judas, 2002; Kostovic and Vasung, 2009; Vasung et al., 2010).

3.4 Microstructure of the cortical plate of the baboon fetal brain

The organized radial organization of the cortical plate reflected by high anisotropic values from DTI has been observed not only in the human fetal brain, but also in the baboon fetal brain. Besides the human fetal brain, the microstructural dynamics of cerebral cortex of different animal models (e.g. Mori et al., 2001; Kroenke et al., 2007; Huang et al., 2008; Kroenke et al., 2009; Takahashi et al., 2011) have been studied with DTI. In a study by Kroenke and colleagues (Kroenke et al., 2007), the cerebral cortex of the baboon fetal brain during gestational development have been characterized with RA measurements derived from DTI. In this study, global RA decrease in the baboon cerebral cortex has been observed, as well as a locally differential RA decrease pattern. Despite that the local anisotropy decrease rate may vary at corresponding cerebral cortical plate regions of human (see 3.1) and baboon fetal brain, two features of microstructural dynamics during cortical plate development, global decrease and locally differential decrease rate, seem preserved across the species.

As an example, the microstructure of the cortical plate the baboon fetal brain at 0.5 term has been characterized with the RA map, as shown in Fig. 6. E90 of Baboon fetal brain is at 0.5 term, equivalent to around 20wg human fetal brain. The Baboon fetal brain at its mid-fetal stage is characterized with high RA values at most of the cortical regions. Maps of RA of E90 baboon brain are shown in coronal slices views from caudal (Fig. 6a) to rostral (Fig. 6e). In the most caudal and rostral slices, RA values are uniformly high (yellow; $RA > 0.6$). In intermediate slices (Figs. 6b–6d), RA values are uniformly high dorsally and laterally, but there is a sharp transition to much lower values ($RA < 0.3$) in a restricted region ventrally and medially, near the junction between frontal and temporal lobes.

The cortical surface of the E90 baboon brain hemisphere intersected with the middle three coronal image slices in the second row of Fig. 6f–6h (enlarged slices) shows the region of low anisotropy in the ventromedial cortex to include classical allocortex plus adjoining periallocortical regions. These changes from high to low anisotropy appears to reflect the boundary between isocortex, defined as the cortex destined to form six layers at maturity, and other cortical regions (proisocortex, periallocortex and allocortex). Despite these changes, most of the cortical plate of the baboon fetal brain at E90 demonstrates a uniform high anisotropy profile. In contrast, the cortical plate of human fetal brain at the 0.5 term (around 19–21 wg) shows a relatively low and inhomogeneous anisotropy profile (Fig. 3).

4. DTI of the white matter of human fetal brain

DTI-derived metrics can be used to assess white matter integrity of the fetal and newborn brains. The measurements of white matter microstructural integrity with DTI have clinical significance which has been demonstrated in the literature (e.g. Huppi et al., 1998; Neil et al., 1998; Huppi et al., 2001a, 2001b; Miller et al., 2002; Mukherjee et al., 2002; Neil et al., 2002) and will not be elaborated below. In the following review on white matter of fetal

brain, the traced individual white matter tracts and brain connectivity based on DTI tractography are focused on in 4.1 and 4.2, respectively.

4.1 Heterogeneous development of different major tract groups

With DTI tractography, three-dimensional reconstruction of traced individual white matter tracts clearly shows the developmental pattern of these tracts. The reconstructed limbic, brain stem, commissural, projection and association white matter tracts of fetal brains at 13, 15 and 19 weeks are shown in Fig. 7 and thalamocortical tracts are shown in Fig. 8 to reveal morphology of individual tracts during development. 13wg to 19wg are an important period in the second trimester of fetal brain development. This period is featured with the formation of some new white matter tracts and dramatic morphological changes of most others. The findings below were based on tractography of DTI of well-fixed fetal brains (Huang et al., 2009; Vasung et al., 2010). The formation of these tracts was also observed by tractography with diffusion MRI data of fixed fetal brains by other investigators (Takahashi et al., 2012). In addition, all the traced fiber tracts have been confirmed with histological images (Bayer and Altman, 2004, 2005).

Limbic tracts (Figs. 7a)—Limbic tracts are among those developing early and before second trimester based on tractography results (Huang et al., 2009) and histological images (Bayer and Altman 2005). By 13 gestational weeks, limbic fibers are visible in DTI color-encoded maps. Limbic tracts include stria terminalis, fornix and the cingulum bundle. Among these tracts, the stria terminalis and the fornix, which are relatively small tracts in adult brains, can be clearly appreciated and traced with DTI tractography at 13 weeks. Meanwhile, the cingulum bundle can be traced only in the fetal brain at 19 weeks. Limbic tracts are those developing earliest.

Tracts in the brain stem (Fig. 7b: green fibers and Fig. 7c: purple fibers)—Tracts in brain stem also develop early. At 13 weeks, the pontine crossing tract and the corticospinal tract can already be identified with DTI color-encoded map (Huang et al., 2009). At 15 weeks, the pontine crossing tract increases its volume and completely surrounds the corticospinal tract with a configuration similar to that of the adult brain. During 15 – 21 weeks, the corticospinal tract increases its size relative to the pons. Simultaneously, the middle and inferior cerebellar peduncles and the medial lemniscus become clearly visible (Huang et al., 2009). From Fig. 7b and 7c, the middle and inferior cerebellar peduncles exist with good shapes from 13 weeks.

Commissural tracts (Figs. 7b)—During the second trimester, major commissural tracts such as corpus callosum become apparent. Among the commissural tracts, the anterior commissure and optic chiasm appear earlier than the corpus callosum (Huang et al., 2009). The corpus callosum appears and is traceable with DTI tractography at 15 weeks (Fig. 7b). Previous investigations (Rakic and Yakovlev, 1986; Ren et al., 2006) also have reported the formation of the corpus callosum at about 15 gestational weeks with histology. After the initial appearance of the corpus callosum, the corpus callosum extends in both anterior and posterior directions during the following weeks. At 19 weeks of gestational age, anterior component of the corpus callosum is dominant (Fig. 7b), indicating that the corpus callosum has a more anterior development from 15 to 19 weeks.

Projection tracts (Figs. 7c)—The internal capsule is recognizable early at 13 weeks and is a good landmark to delineate the ganglionic eminence and caudate nucleus from the putamen and globus pallidus (Huang et al., 2009). Throughout development, the internal capsule seems to extend from its core to more anterior and posterior areas (Fig. 7c).

Projection fibers can be delineated at 13 weeks, and develop to its peripheral regions during the second trimester.

Association tracts (Figs. 7d)—Some association tracts, specifically uncinate and inferior longitudinal fasciculi, become apparent at the age of 15 weeks, and no association fibers can be identified in the 13-week brain (Huang et al., 2009). The reconstructed sagittal striatum and the external capsule from 13 to 19 weeks are shown in Fig. 7d. The initial formation of these different fiber tracts occurs at different stages. The external capsule, uncinate fasciculus and inferior longitudinal fasciculus appear at 15 weeks (Huang et al., 2009). With DTI-based tractography, the external capsule can be traced in early second trimester brains at 13 or 15 weeks (Fig. 7d), but the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus or uncinate fasciculus cannot be traced in these brains. For 19 week brains, the inferior fronto-occipital fasciculus, inferior longitudinal fasciculus and uncinate fasciculus can be traced, shown as Fig. 7d. Unlike the corpus callosum or internal capsule, these association fibers do not undergo significant development during the second trimester. It is worth noting that the superior longitudinal fasciculus is not detectable in the fetal brain of second trimester with the current image resolution. This tract is closely related to evolution and is a unique white matter tract well developed in human brain in comparison to the monkey and Chimpanzee brain (Rilling et al., 2008). As reported in previous studies (Huang et al., 2006; Zhang et al., 2007), the superior longitudinal fasciculus is not prominent, even at birth.

Thalamocortical tracts—With DTI tractography, thalamocortical pathways can be traced within the anterior, superior and posterior thalamic radiations (Fig. 8a–8b). Fig. 8c shows that among the traced thalamocortical tracts, very few individual fiber bundles reach the subplate. In a series of studies using AChE (Acetylcholinesterase) histochemistry, Kostovic and colleagues (Kostovic and Goldman-Rakic, 1983; Krmpotic-Nemanic et al., 1983; Kostovic, 1990a,b; Kostovic and Rakic, 1990) have shown that thalamocortical fibers accumulate in the superficial subplate after a prolonged ‘waiting’ period. This event occurs almost simultaneously in neocortical regions. These properties can be more clearly demonstrated with the histological images Fig. 8e and Fig. 8f. The developmental shifts in histochemical properties and fibrillar organization (Fig. 8e) within the subplate show that this cellular compartment permanently changes in its fibrillar and cellular content. The intense AChE reactivity of the thalamocortical growing front in the superficial subplate shown in Fig. 8f and marked with an “x” is enhanced due to the overlap with AChE staining in “cholinergic” fibers from the magnocellular basal forebrain (Kostovic, 1986). The hydrophilic extracellular matrix-rich zone of the subplate can be visualized in conventional T2-weighted magnetic resonance images as high intensity zones beneath the cortical plate, as shown in Fig. 8d. The proper time of ingrowth of thalamocortical fibers into the cortical plate is crucial for normal maturation and the formation of functional cerebral cortical areas (Rakic et al., 1991). Similarly, the appearance of primary gyri and sulci within the human fetal brain coincides with the thalamocortical axonal ingrowth (Kostovic and Vasung, 2009). Furthermore, the ‘transient synaptic circuitry’ within the subplate zone and the proper expression of extracellular axonal guidance molecules play vital roles for proper functional and architectural maturation of the cerebral cortex that serves the most complex cognitive skills (Kostovic and Judas, 2006).

The visibility of different white matter tracts is affected by the factors such as image resolution. *Ex vivo* DTI data shown in Fig. 7 and Fig. 8 have the resolution from 200 to 400µm. Most fibers in the cerebrum, with a pronounced appearance, can be revealed with the *ex vivo* DTI data set. However, some fibers, such as superior cerebellar peduncle, can be seen in histological images (Bayer and Altman, 2005), but not in DTI images. Therefore, it

is possible that some existent fibers could not be identified in DTI images with the current resolution.

To summarize the differential developmental pattern of individual white matter tracts, limbic fibers develop first, association fibers last and commissural, thalamocortical and projection tracts form from the core to the periphery of the brain. The developmental sequence of white matter fibers observed with DTI tractography above coincides with the report of Partridge and colleagues (Partridge et al., 2004) with FA and ADC measurements of preterm newborn brains.

4.2 Fetal brain connectivity revealed with diffusion MR tractography

The tractography based on diffusion MR data has also made it possible to reveal the fetal brain connectivity. Different from 4.1 where regions of interests (ROIs) were carefully selected to trace certain white matter tracts, in this session, all fibers of a whole brain or all fibers passing through an axial/coronal/sagittal slice were traced to reveal brain connectivity. As shown in Fig. 9, from 19wg to 42wg of fetal brain development, regression of initial radial organization in the cerebral wall and sequential emergence of intracerebral connectivity is apparent (Takahashi et al., 2012). In addition, there is regression of tangential connectivity in the ganglionic eminence (Huang et al., 2006; Takahashi et al., 2012). This order of regional radial and tangential regression and emergence of intracerebral connectivity follows the same order of normal gyrification (White et al., 2010)

In Fig. 9a, the 19wg fetal brain is characterized with the dominant radial pathways with immature forms of projection, limbic and few emergent association pathways. Similar white matter fiber pattern can also be observed in Fig. 7 for the fetal brain at 19wg. In Fig. 7, the white matter fibers were categorized by functionally unique tract groups, while all tractography fibers touching a sagittal image slice are combined and demonstrated Fig. 9. Tractography at 22wg fetal brain still shows dominant radial pathways. In addition, long range connectivity underlined by long range association fibers appears (Fig. 9b). In Fig. 9c, the 26 wg brain has less predominant radial pathways in dorsal frontal, parietal and inferior frontal lobes and emergent short range and long range association fibers. In 33wg fetal brain, the disruption of the radial pathways extends to temporal and occipital lobes with the concurrent appearance of more short and long range association tracts (Fig. 9d). 42wg fetal brain is around term. At this stage, radial pathways have almost disappeared. Instead, complex and abundant short and long range association fibers become dominant (Fig. 9e).

Recent study by Wedeen et al. (Wedeen et al., 2012) revealed the geometric structure of the brain pathways while Zilles and Amunts (Zilles and Amunts, 2012) suggest that the wiring of the brain follows the patterning of the cortex. Therefore, the investigations of wiring dynamics of the human brain pathways together with the changes in cortico-areal metrics during early human brain development can provide insight on interactions of these developmental processes.

5. Conclusions

Brief concepts of DTI and DTI-based tractography, structural characterization of the fetal cortex and other components of fetal cerebral wall with both DTI and histology, dynamics of white matter tract growth and the brain connectivity patterns during the fetal development with DTI tractography have been introduced. FA and RA derived from DTI provide a quantitative way to delineate the heterogeneous cortical microstructural changes during human and baboon fetal development. For human fetal brain from 13wg to 21wg, different neocortical areas are featured with unique pattern of anisotropy decrease which is mainly caused by a distinct pattern of disruption of radial organization and dendritic growth.

Histological imaging with different staining, complementary to DTI measurements, revealed radial and tangential organizations in the cerebral wall layers other than cortical plate. DTI tractography depicted differential development of different white matter tracts with limbic tracts developing earliest and long association tracts latest. A whole brain tractography further provided the developmental pattern of brain connectivity from 19wg to 42wg. During this stage, there is a regional regression of radial organization and regional emergence of fetal brain connectivity in general from posterodorsal to anteroventral with local variations. There are many transient structures which are unique in the fetal stage and disappear at term. These unique structures, such as the subplate and ganglionic eminence, are usually associated with a specific neurodevelopmental processes. Future advances of DTI technology, combined with histology, will undoubtedly enhance our understanding of the early brain development.

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Highlights

Fetal cerebral wall and white matter have been delineated with DTI and histology.
DTI and histology characterize the cerebral wall at macroscopic and microscopic level.
Microstructural dynamics of fetal cortical development was quantified by DTI.
Differential fetal white matter growth pattern was revealed with DTI tractography.
Patterns of developmental fetal brain connectivity were reviewed.

Fig. 1.

Striking morphological changes from the 4th wg to the 40th wg. The reconstruction of the 4th wg is adapted from the textbook (Chapter 2 of Nolte, 1999). Many questions on micro- and macroscopic structural changes from a “tubular” structure at the 4th wg to an extremely complicated human brain at birth remain unanswered. The pink color indicates the cerebrum or corresponding prosencephalon at earlier stage. The light purple color indicates the brain stem and cerebellum or corresponding rhombencephalon at earlier stage.

(a) Diffusion MRI data acquisition **(b) Diffusion tensor fitting** **(c) DTI-derived maps** **(d) DTI-based tractography** **Figure 2.**

Outline of DTI data acquisition, tensor fitting, DTI-derived quantification maps and DTI-based tractography of an *in vivo* preterm neonate brain at 32 weeks of gestation.

Figure 3.

Coronal images of FA map (upper row) and mapping of FA on the cortical surface (middle row) of 13wg, 15wg, 17wg, 19wg and 21wg fetal brain from left to right. The three layers, marked with “1”, “2” and “3”, can be clearly differentiated from all cerebral wall of 13wg to 21wg fetal brains with FA contrasts. Layer 1, 2 and 3 indicate cortical plate, subplate and inner layer (the cerebral wall compartment between ventricle and subplate), respectively. The heterogeneous FA decrease patterns of three representative cortical regions, VFC, S1C and IPC, are shown in the lower row. The decrease line in the lower row is averaged from 11 cortical ROIs (Huang et al., 2013) covering major cortical areas across the cortical plate. The locations of these three ROIs are labeled in 21wg cortical FA map in the middle row.

Figure 4.

GFAP histological (a, b) image of a 17wg fetal brain and the corresponding FA maps (c, d) are shown in the upper panel. The close-to-ventricle part of inner layer (layer 3) has clear radial fibers in GFAP images (a, b). Neurofilament histology image of 16wg fetal brain (e, f) and corresponding FA maps (g, h) are shown in the lower panel. Tangential fibers can be observed in the close-to-subplate part of inner layer (layer 3). The ROIs for FA measurements in (i) are shown in (d) and (h) as dashed boxes, which are consistent with those derived from histology contrasts in (b) and (f), respectively. The close-to-ventricle part has more uniformly distributed radial fibers and hence has a higher FA value than close-to-subplate region where tangential and radial fibers may cross to each other. Yellow lines in (b) and (f) indicate the orientations of the microstructures. Green lines in (b) point to the region where GFAP stain color changes and possibly the crossing of tangential and radial fibers takes place. Asterisk in (i) indicates p less than 0.001. Adapted with permission from Huang et al., 2012.

Figure 5.

Maps of primary diffusion eigenvectors overlaid on maps of apparent diffusion coefficient maps from infants of 26 and 35 wg (a), diagrams depicting the ordered radial structures in the cerebral layer of 26 week brain (b) and 35 week brain (c). In the left image of (a), the directions of the vectors are oriented radially in cortex at 26 week of gestational age. By 35 weeks of gestational age, the radial structure is much less evident, shown in the right image of (a). At 26 week of gestational age (b), radial glial fibers and pyramidal neurons with prominent, radially oriented apical dendrites are shown. This organization has the effect of restricting water displacement parallel to the cortical surface more than displacement orthogonal to it, resulting in diffusion ellipsoids which are non-spherical with their major

axes oriented radially, indicated by arrows. By 35 weeks of gestational age (c), prominent basal dendrites for the pyramidal cells and thalamocortical afferents have been added. This has the effect of restricting water displacement more uniformly in all directions. As a result, the diffusion ellipsoids are spherical, without a preferred orientation. Adapted with permission from McKinstry et al. 2002.

Figure 6.

Isocortical versus allocortical diffusion anisotropy of an E90 baboon fetal brain. Coronal slices (a–h) of RA maps are shown for locations indicated on the surface models (i,j,k). Coronal slices f–h are enlargements of slices b–d to show details. The classification of surface structures used is illustrated on slices f–h. Isocortex is shown in blue, “other cortex” (including allocortex) in green, unassigned cortex in brown, and the “medial wall” (not containing cortex) in gray. The anisotropy color scale for “volume data” (a–h) differs from the surface anisotropy color scale (i–l); however, in both cases, yellow is most anisotropic. At the location of the isocortex/allocortex boundary, a dramatic change in cortical diffusion anisotropy (high-isocortex/low-allocortex) is observed. In the ventral view inset (l), a color

code is used to show the location of proisocortical areas lai, lam, and 13a described by Ferry et al. (2000) using surface registration to the adult macaque atlas surface. Adapted with permission from Kroenke et al., 2007.

Figure 7.

Three-dimensional depiction of developmental white matter fibers. (a) is a lateral view of limbic tracts where pink fibers in 13, 15 and 19 week brains are the fornix and stria terminalis and purple fibers in 19 week brain indicate the cingulum bundle. (b) is an oblique view of commissural fibers where pink and green fibers in 13, 15 and 19 week brains are the corpus callosum and the middle cerebellar peduncle. (c) is a lateral view of projection fibers where red and purple fibers in 13, 15 and 19 week brains are the cerebral peduncle and the inferior cerebellar peduncle, respectively. (d) is a lateral view of association tracts where blue fibers in 13 and 15 week brains are the external capsule and green and red fibers in a 19 week gestation brain are inferior longitudinal fasciculus/inferior fronto-occipital peduncle

and uncinatus fasciculus, respectively. For anatomical guidance, thalamus (yellow structure in a,b,c,d) and ventricle (gray structure in a,c,d) are also shown. Adapted with permission from Huang et al., 2009.

Figure 8.

Development of thalamocortical fibers at mid-fetal period. At 21wg fibers from different thalamic nuclei/complex (ROI, b) are taking different routes (anterior, superior, inferior and posterior thalamic radiation) towards the “waiting compartment” - subplate (a) and cortical plate. In c the waiting fiber bundles in subplate, originating from thalamus can be seen by DTI at 26wg. Accumulation of the growing front of thalamocortical fibers in superficial subplate is shown by AChE staining (f, X) embedding in extracellular matrix rich neuropil shown by fibronectin staining (e, X). In vivo T2 weighted image (d) are also useful in showing subplate part (d, +) due to the different T2 properties of the fetal laminae. Hippocampus and the enlarged marginal zone are showing similar T2 tissue properties in *in vivo* T2 weighted image (d, curved arrows). sp, subplate; ci, internal capsule. Adapted with permission from Vasung et al., 2010.

Figure 9.

Tractography pathways at 19wg (A), 22wg (B), 26wg (C), 33wg (D) and 42wg (E). (A) Tractography at 19wg shows dominant radial pathways with immature forms of projection, limbic, and few emergent association pathways. (B) Tractography at 22wg shows dominant radial pathways and emerging long-range connectivity patterns. (C) Tractography at 26wg shows less predominant radial pathways in dorsal frontal, parietal and inferior frontal lobes, and emergent short-range corticocortical and long-range association pathways. (D) Tractography at 33wg shows less dominant radial pathways in the temporal and occipital lobes, emergent short-range corticocortical and long-range association pathways ventrally. (E) Tractography at 42wg shows no evident radial pathways, abundant complex, crossing short- and long-range corticocortical association pathways. Small letters in all panels indicate individual identifiable tracts and the names can be found in the legend of figure 2 in Takahashi et al., 2012. In all panels, tractography pathways passing through a sagittal slice in upper left corner is shown. In a coronal slice next to the upper left corner sagittal slice, the locations of the sagittal slice is shown as a yellow line. Only 70% of the tractography fibers that touched each sagittal slice and were more than 2mm in length were shown. Colors

indicate orientation of the pathways. Red: left-right, blue: anterior-posterior, and green: dorsal-ventral orientation. Adapted with permission from Takahashi et al., 2012.