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Development of cerebellar connectivity in human fetal brains revealed by high angular resolution diffusion tractography

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ABSTRACT

High angular resolution diffusion imaging (HARDI) tractography has provided insights into major white matter pathways and cortical development in the human fetal cerebrum. Our objective in this study was to further apply HARDI tracography to the developing human cerebellum ranging from fetal to adult stages, to outline in broad strokes the 3-dimensional development of white matter and local gray matter organization in the cerebellum. We imaged intact fixed fetal cerebellum specimens at 17 gestational weeks (W), 21W, 31W, 36W, and 38W along with an adult cerebellum for comparison. At the earliest gestational age studied (17W), coherent pathways that formed the superior, middle, and inferior cerebellar peduncles were already detected, but pathways between deep cerebellar nuclei and the cortex were not observed until after 38W. At 36–38W, we identified emerging regional specification of the middle cerebellar peduncle. In the cerebellar cortex, we observed disappearance of radial organization in the sagittal orientation during the studied developmental stages similar to our previous observations in developing cerebral cortex. In contrast, in the axial orientation, cerebellar cortical pathways emerged first sparsely (31W) and then with increased prominence at 36–38W with pathways detected both in the radial and tangential directions to the cortical surface. The cerebellar vermis first contained only pathways tangential to the long axes of folia (17–21W), but pathways parallel to the long axes of folia emerged between 21 and 31W. Our results show the potential for HARDI tractography to image developing human cerebellar connectivity.

Introduction

The maturation of the human cerebellum is more protracted than that of the cerebrum and continues through the first postnatal year (Altman and Bayer, 1997; Saksena et al., 2008; Wang and Zoghbi, 2001). Like the cerebral cortex, the morphogenesis of the cerebellar cortex is characterized by phases of neuronal proliferation, migration, differentiation, axon growth, synaptogenesis, and pruning (Catz et al., 2008; Lavezzi et al., 2006; Sidman and Rakic, 1973; Wang and Zoghbi, 2001). Cerebellar development is distinct, however, in that granule cell precursors migrate in the reverse direction from the external granular layer inward past the molecular layer and Purkinje dendrites and somas, and the human cerebellar cortex is composed at first of two layers, then three, five, four, and finally after birth acquires the adult three layered pattern (Rakic and Sidman, 1970). In the cerebellar cortex, Bergmann glial fibers provide the radial guidance for this migration (Sidman and Rakic, 1973).

Coincident with the formation of these fiber pathways is the process of axonal myelination which starts well before birth in humans, and, depending on location in the brain, continues into adulthood (Brody et al., 1987; Yakovlev and Lecours, 1967). Cerebellar white matter does not myelinate uniformly, but along a temporal gradient commencing with the archicerebellum, and followed by the white matter of the paleocerebellum and neocerebellum (Brody et al., 1987; Gilles et al., 1976). Myelination in cerebellar white matter pathways begins during the third trimester and continues after birth. Myelination in the middle cerebellar peduncle (MCP) begins some weeks later, around the time of birth (Brody et al., 1987; Yakovlev and Lecours, 1967).

These myelination processes have been demonstrated in pathological specimens derived from human fetuses (Chong et al., 1997; Triulzi et al., 2005, 2006). They have also been shown using conventional MRI techniques (Barkovich et al., 1988; Van der Knaap and Valk, 1990; for review Paus et al., 2001). Diffusion tensor imaging (DTI), based on measurement of the directional bias of water molecule diffusion in brain tissue (Basser et al., 1994) and associated post-processing data reconstruction using

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tractography techniques (Conturo et al., 1999; Jones et al., 1999; Mori et al., 1999), permits examination of white matter axonal organization running in many directions throughout the entire brain *in vivo*. Our understanding of developing human cerebellar organization lags behind the cerebrum, however, because with DTI tractography it is particularly difficult to resolve the 3-dimensional geometry of the cerebellar folia and associated connectivity.

Recently, high-angular resolution diffusion imaging (HARDI) has been shown to improve the characterization of complex tissue coherence compared to DTI, by defining a fiber orientation distribution function. This approach improves the ability to resolve different diffusion directions within the same voxel that result from crossing axonal bundles (Leergaard et al., 2010; Tuch et al., 2003). HARDI has been effective for delineating tissue coherence associated with the structural changes that occur in developing fetal (preterm) brains, in which the process of migration and myelination is incomplete.

A number of DTI studies on fetal and newborn human cerebrum (Baratti et al., 1999; Huppi et al., 1998; Neil et al., 1998; Rutherford et al., 1991; Sakuma et al., 1991; for review Gupta et al., 2005; Huang et al., 2009 ;Neil et al., 2002; Prayer et al., 2006; Rollins, 2007) as well as cerebellum (Huang et al., 2009; Saksena et al., 2008) have reported that FA values increase in the white matter with age. Some investigators have also performed DTI tractography in developing human brains including fetal and preterm brains (Bassi et al., 2008; Berman et al., 2005; Bui et al., 2006; Huang et al., 2006, 2009; Kasprian et al., 2008; Saksena et al., 2008; Tam et al., 2009) showing the development of major white matter pathways. We have applied HARDI tractography to immature cat and human cerebrum to provide whole-brain 3-dimensional visualization of developing fiber systems (Takahashi et al., 2010, 2011, 2012). However, to date there has been no HARDI tractography study of the developing human cerebellum. Our objective in this study was to apply HARDI tractography to developing human cerebellum during fetal and adult ages, to explore the 3-dimensional development of white matter and local gray matter pathways in the cerebellum.

Experimental procedures

Datasets

We used human fetal cerebella at 17 gestational weeks (W), 21W, 31W, 36W, and 38W (Engle, 2004), as well as adult cerebella (eight samples in total; two samples at 17W and two samples in adult). The cerebella were obtained from the Brigham and Women's Hospital Department of Pathology, under protocols approved by the hospital's institutional review board for human research. They include specimens from terminations, stillbirths, and neonatal deaths, submitted for pathologic examination after consent of parent(s) or guardian(s). A perinatal neuropathologist studied each sample at the time of post-mortem examination, and only those tissues not needed for immediate diagnosis were fixed in 4% paraformaldehyde and submitted for coded (de-identified) specimen scanning (mean fixation period was around 2–3 months). Any cases with known or suspected malformations, disruptions, or other lesions (on basis of in utero ultrasonography, or post-mortem findings) were excluded from this study. Cerebella were removed from the cranium and fixed in a 4% paraformaldehyde solution containing 1 mM gadolinium (Gd-DTPA) MRI contrast agent for at least 1 week to reduce the T1 relaxation time while ensuring sufficient T2-weighted signal remains. During image acquisition, the brains were placed in Fomblin solution (Ausimont, Thorofare, NJ).

Scanning parameters

The pulse sequence used for HARDI acquisition was a 3D diffusion-weighted spin-echo echo-planar imaging (EPI) sequence (Takahashi et al., 2012, 2013; Xu et al., 2014), TR/TE 1000/40 ms, with an imaging matrix of 112 × 112 × 128 pixels. Sixty diffusion-weighted measurements ($b = 8000$ s/mm^2) and one non-diffusion-weighted measurement ($b = 0$ s/mm^2) were acquired at a 4.7T Bruker Biospec MR system with $\delta = 12.0$ ms, $\Delta = 24.2$ ms. Spatial resolution was 415 × 500 × 550 μm for 17–22W, 525 × 525 × 600 μm for 31W, 700 × 830 × 860 μm for 36–40W, and 525 × 525 × 600 μm for adult specimens. The adult specimens were scanned as hemicerebella, after sectioning in the midsagittal plane. The total acquisition time was approximately 1 h and 50 min for each imaging session.

Diffusion data analyses

Diffusion Toolkit and TrackVis (http://trackvis.org) were used to reconstruct and visualize tractography pathways. The color-coding of tractography pathways is based on a standard RGB code, applied to the vector between the end-points of each fiber. We used a streamline algorithm for diffusion tractography (Mori et al., 1999) described in previous publications (Takahashi et al., 2011, 2012). The term "streamline" refers to the fact that we connect tractography pathways using local maximum or maxima. This is true for both DTI and HARDI. The streamline technique is limited in its ability to resolve crossing pathways when used with the traditional DTI technique, because one simply connects the direction of the principal eigenvector on a tensor to produce the DTI tractography pathways. This is a recognized limitation of DTI, as discussed in the DTI paper of Mori (1999). For this reason, in the current study, we used HARDI, which detects multiple local maxima on an ODF (orientation distribution function). We used all the local maxima to produce HARDI tractography pathways, thus enabling us to identify crossing pathways within a voxel.

Trajectories were propagated by consistently pursuing the orientation vector of least curvature. We terminated tracking when the angle between two consecutive orientation vectors was greater than the given threshold (40°). As in previous studies, no FA threshold was applied (e.g. Takahashi et al., 2010; Vishwas et al., 2010). In many tractography studies, fractional anisotropy (FA) values are used to terminate fibers in the gray matter, which in adults has lower FA values than the white matter. However, as one of the objectives of our study was to detect fibers in low FA areas where myelination is not complete, we used brain mask volumes to terminate tractography fibers instead of the FA threshold (Takahashi et al., 2010). This method has been used previously (Takahashi et al., 2011) and is an acceptable alternate method (Schmahmann et al., 2007; Wedeen et al., 2008).

Results

Improved tractography results using a size-optimized MR coil for the cerebellum

Signal-to-noise ratio and tractography outcomes significantly improved using a size-optimized RF coil for each sample (Fig. 1). The cerebellum with the cerebrum attached was scanned using a large coil for a whole brain (Fig. 1, upper row), which produced many short irregular non-anatomic tractography pathways that are inconsistent without knowledge of cerebellar anatomy. We then used a size-optimized coil for the cerebellum after dissecting the cerebellum from the specimen, and obtained longer more coherent tractography pathways due to the higher signal-to-noise ratio and spatial resolution (Fig. 1, lower row).

Cerebellar white matter pathway development

The corticospinal tracts and medial lemniscus were readily identified along with the superior, middle, and inferior cerebellar peduncles at 17W (Fig. 2A–C). The tracks passing through the MCP did not reach the cerebellar cortex, but projected to broad areas in the cerebellar hemisphere, terminating deep to the cerebellar cortex (Fig. 2D, E).