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Functional correlates of central white matter maturation in perinatal period in rabbits

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ABSTRACT

Anisotropy indices derived from diffusion tensor imaging (DTI) are being increasingly used as biomarkers of central WM structural maturation, myelination and even functional development. Our hypothesis was that the rate of functional changes in central WM tracts directly reflects rate of changes in structural development as determined by DTI indices.

We examined structural and functional development of four major central WM tracts with different maturational trajectories, including internal capsule (IC), corpus callosum (CC), fimbria hippocampi (FH) and anterior commissure (AC). Rabbits were chosen due to perinatal brain development being similar to humans, and four time points were studied: P1, P11, P18 and adults. Imaging parameters of structural maturation included fractional anisotropy (FA), mean and directional diffusivities derived from DTI, and T2 relaxation time. Axonal composition and degree of myelination were confirmed on electron microscopy. To assess functional maturation, conduction velocity was measured in myelinated and non-myelinated fibers by electrophysiological recordings of compound action potential in perfused brain slices.

Diffusion indices and T2 relaxation time in rabbits followed a sigmoid curve during development similar to that for humans, with active changes even at premyelination stage. The shape of the developmental curve was different between the fiber tracts, with later onset but steeper rapid phase of development in IC and FH than in CC. The structural development was not directly related to myelination or to functional development. Functional properties in projection (IC) and limbic tracts (FH) matured earlier than in associative and commissural tracts (CC and AC).

The rapid phase of changes in diffusion anisotropy and T2 relaxation time coincided with the development of functional responses and myelination in IC and FH between the second and third weeks of postnatal development in rabbits. In these two tracts, MRI indices could serve as surrogate markers of the early stage of myelination. However, the discordance between developmental change of diffusion indices, myelination and functional properties in CC and AC cautions against equating DTI index changes as biomarkers for myelination in all tracts.

Introduction

The advantages of MRI being a non-invasive tool for dynamic measurement of longitudinal brain development has spurred the application of advanced imaging techniques in large cohorts of infants and children, and the development of novel analytical frameworks to distinguish normal and abnormal brain development (Dean III et al., 2014; Dubois et al., 2013; Englander et al., 2013; Prastawa et al., 2010; Vasung et al., 2013). Diffusion tensor imaging (DTI) provides important quantitative indices of anisotropic water diffusion in white matter (WM) tracts that evolve with development (Huppi et al., 1998; Mukherjee et al., 2001; Neil et al., 1998) and reflect changes in structural composition and possibly functional properties (Dubois et al., 2008b) during fiber tract maturation. The origin of diffusion anisotropy in WM is attributed to the ordered arrangement of axonal membranes, neurofilaments, microtubules and myelin sheaths (Beaulieu, 2002), but little work has been done to understand the relative contribution of the various structural elements and tissue compartments in hindered and restricted diffusion (Assaf and Basser, 2005; Ben Bashat et al., 2005), especially during perinatal development.

Fractional anisotropy (FA) and directional diffusivity changes are being increasingly used to diagnose early motor and cognitive abnormalities in neonates and infants (Anjari et al., 2007; Dubois et al., 2013; Rose et al., 2007; van der Aa et al., 2013; van Kooij et al., 2012). WM tracts are substantially anisotropic in diffusion weighted images even in fetuses and neonates long before the onset of myelination

Abbreviations: CP, cerebral palsy; DTI, diffusion tensor imaging; FA, fractional anisotropy; ADC, apparent diffusion coefficient; EM, electron microscopy; WM, white matter; CC, corpus callosum; AC, anterior commissure; IC, internal capsule; FH, fimbria hippocampi; CV, conduction velocity; CAP, compound action potential.

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(Drobyshevsky et al., 2005; Neil et al., 1998; Wimberger et al., 1995). FA increases with development, with contribution from changes in both radial diffusivity and axial diffusivity (Gao et al., 2009; Geng et al., 2012; Partridge et al., 2004). While myelination is not necessary to the origin of diffusion anisotropy in WM (Beaulieu, 2002), it may contribute to the further decrease of radial diffusivity by the increase of cellular barriers for water diffusion as demonstrated in genetic (Gulani et al., 2001; Song et al., 2002) or induced (Thiessen et al., 2013) demyelination adult animal models. It has been suggested that abnormally high radial diffusivity in the posterior limb of internal capsule may represent delayed myelination in human infants (Cowan and de Vries, 2005). But, histological validation of such observations has been mostly restricted to myelin basic protein or axonal immunohistochemical markers in animal models (Drobyshevsky et al., 2007; Jito et al., 2008; Cengiz et al., 2011; Calabrese and Johnson, 2013).

Understanding the extent of contribution of myelination to the WM anisotropy indices in infant development would help establish the timing and extent of diffusion anisotropy loss as a marker of delayed myelination. On the other hand it is important to see whether changes in directional diffusivities corresponds to appearance of fast conducting myelinated axons (Drobyshevsky et al., 2005) as this may provide a biomarker of functional maturation of WM tracts (Dubois et al., 2008b). The relationship between functional maturation, reflected by changes in conduction velocities and compound action potential (CAP) properties and structural maturation, reflected by changes in directional diffusivities and underlying axon density and composition, may vary during developmental trajectory in different WM (Dubois et al., 2008a).

Taking advantage of the fact that different WM tracts have different developmental trajectories we investigated structural–functional relationship of WM in major central tracts. We included projection, commissural, associative and limbic tracts in our study of perinatal development from neonates to juvenile rabbit kits. What is unique with this study is the special emphasis made on accurate co-registration of MRI, functional and ultrastructural measurements. The main focus of the study was on the internal capsule (IC) since perinatal injury to cortico-spinal projections is correlated with motor deficits in humans (Rose et al., 2007) and in our animal model of global antenatal hypoxia–ischemia (Drobyshevsky et al., 2007). IC is implicated as the region where major etiopathogenetic events of human cerebral palsy (CP) take place (Laura and Deborah, 2010; Sanger, 2003).

Methods

General plan of experiments

New Zealand White pregnant rabbits (Myrtle's Rabbits, TN) were allowed to deliver in a nest box at term (31.5 days). Rabbit kits were fed by dam and allowed to grow till P18. Newborn rabbit kits are rather immature at birth in their sensory and motor development (Hudson and Distel, 1986). Rabbit kits are born naked, with eyes not open, outer ears not developed, and poor motor coordination. By day 7 they are capable of limited oriental response to auditory stimuli and begin to open their eyes on day 9 or 10. Kits start to leave the nest when 13 to 18 days old, by which time they are able to maintain a stable body temperature and have much improved motor coordination. New Zealand rabbits sexually mature at 6 months. The study followed a longitudinal design, starting from postnatal age day 1 (P1), when kits exhibit poor motor abilities and myelination has not yet begun, through P11, when kits open their eyes and show more motor abilities, to P18 when kits show adult-like motor abilities and myelination is at an advanced stage. Rabbit dams were processed to obtain adult data.

At P1, P11 and P18, the kits underwent serial in-vivo MRI examination. After each MRI session at P1, P11 and P18, brains from a subset of kits were extirpated for electrophysiological recordings, followed by electron microscopy. The methodology of the study was focused to reveal intricate connection between changes in microstructural organization of the maturing central WM and functional properties of the tracts. We put special emphasis to co-register structural and functional measurements, taking into account the different spatial scales of the techniques such as DTI, electron microscopy and localized CAP recording on brain slices. The order of studies and sampling procedures was arranged so that the measurement sites in WM were co-registered for MRI first, then electrophysiology and histological examinations were conducted using anatomical landmarks (Fig. 1).

MRI methods

Rabbit kits were sedated with i.m. injection of a mixture of Ketamine (35 mg/kg), Xylazine (5 mg/kg), and Acepromazine (1.0 mg/kg). The animals were placed supine in a cradle heated with a water blanket at 35 °C and imaged in a 9.4 T Bruker Biospec system (Bruker, Billerica, MA). The receiver coil was a standard linear Bruker rat brain size coil allowing full brain coverage in P1–P18 rabbits. Number of kits was 11 for P1, 8 for P11, 8 for P18. Three rabbit dams were imaged in the same magnet using 50 mm transceiver surface coil. A modified futility design was used to calculate the number of animals in order to detect a difference of between P1 and P11 of a difference = S.D. with alpha = 0.05 and power = 0.85.

DTI experiments consisted of 15 non-collinear directions diffusion weighted images with TR/TE/NEX 2500/26/4 matrix 128 × 64, zero-padded to 128 × 128, $\delta = 5$ ms, $\Delta = 15$ ms, with b = 0 and 0.8 ms/μm^2. The brain was divided into 12 oblique coronal brain slices, positioned on a sagittal localizer scan in a way that the fifth slice was on anterior commissure and the last slice was crossing anterior edge of superior colliculus. The number of slices was kept constant for all age groups to cover the same area of cerebrum. Slice thickness was therefore variable across individual and age groups and was about 1 mm for P1 and P11, 1.2 mm for P18 kits, and 1.5 mm for adults. In-plane resolution after interpolation was 0.156 mm for P1 and 0.195 mm for P11 and P18 kits. To ensure reproducible pitch angle on oblique coronal imaging sections, slices were oriented orthogonal to frontal cortex pole–pons plane as determined with the aid of multi-slice sagittal localizer scan (Fig. 1). Diffusion tensor was calculated using multivariate linear fitting of signal attenuation from the acquired diffusion weighted images (Basser and Jones, 2002). Apparent diffusion coefficient (ADC), axial (first eigenvalue of the diffusion tensor) and radial (average of the second and third eigenvalues) diffusivities (Song et al., 2002), and fractional anisotropy (FA) maps were calculated (Basser et al., 1994) using in-house software written on MATLAB (MathWorks, Natick, MA).

T2 relaxation time measurement was performed using spin echo multi-echo sequence with TR of 4000 ms and 8 echo times varying from 20 to 160 ms with the same geometry parameters as in DTI experiment. T2 maps were obtained by fitting mono-exponential signal decay curve in the T2-weighted images with different echo times.

High resolution sagittal T2-weighted images (RARE sequence, TR/TE/NEX 4000/90/6 matrix 256 × 128, zero-filled to 256 × 256, 1 mm slice thickness) were obtained to measure cross section areas of the commissural fiber tracts.

Region of interest analysis

Regions of interest (ROIs) of internal capsule (IC), fimbria hippocampi (FH), anterior commissure (AC) and corpus callosum (CC) were outlined using semi-automated routine written in MATLAB. Single seeding voxel was manually selected by clicking on a structure of interest on a directionally color encoded FA maps (Pajevic and Pierpaoli, 1999), by an observer blinded to group assignment. ROIs were systematically placed on 5th slice for AC, 7th slice for FC, 8th slice for IC and CC in rostro-caudal direction (Fig. 1D). The ROI boundaries were outlined by a region growing procedure on a single slice using FA and fiber orientation (Sun et al., 2003). Empirically the chosen cutoff values of 0.3 for