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In vivo evaluation of optic nerve development in non-human primates by using diffusion tensor imaging

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ABSTRACT

Developmental abnormalities of optic nerve are the leading cause of child blindness. The goal of this study was to use diffusion tensor imaging (DTI) to characterize the optic nerve development of non-human primates during the normal maturation from birth to adulthood. Forty healthy rhesus monkeys aged from 2 weeks to 6 years old were scanned with a clinical 3 T scanner. It was demonstrated that the DTI parameters followed an exponential pattern during optic nerve maturation. The time constants of mean diffusivity (MD), fractional anisotropy (FA), axial diffusivity ($\lambda_{\parallel}$) and radial diffusivity ($\lambda_{\perp}$) were 16, 14, 18 and 15 months in rhesus monkeys, respectively. Significant decrease in RD was observed firstly at 12 months after birth ($p < 0.05$). No significant differences were observed between the left and right optic nerves in any age group. The in vivo imaging results reveal the normal evolution patterns of DTI parameters during optic nerve maturation in primates. The data might be used as a reference in the examination of optic nerve developmental abnormalities or injury in children or preclinical studies.

1. Introduction

Optic nerve is part of the central nervous system (CNS) and the pathway to transfer visual information from retina to brain. Optic nerve disorders during the developmental period may lead to partial or complete loss of sight. Developmental anomalies of optic nerve are the leading cause of infant blindness (Garcia-Filion et al., 2008). Also, damaged optic nerve can result in glaucoma and may cause photophobia, lacrimation or blepharospasm (deLuise and Anderson, 1983; Ho and Walton, 2004; Beck, 2001).

Diffusion tensor imaging (DTI) (Le Bihan et al., 2001; Zhou, 2004) is a non-invasive magnetic resonance imaging (MRI) technique and has been used widely to investigate white matter development and integrity in human brains (Alexander et al., 2007; Hermoye et al., 2006; Huppi and Dubois, 2006; Pavuluri et al., 2009; Thomason and Thompson, 2011). Fractional anisotropy (FA) and mean diffusivity (MD) are general measures used to quantitatively characterize the microstructural changes in white matter fiber bundles. Meanwhile, axial diffusivity ($\lambda_{\parallel}$) and radial diffusivity ($\lambda_{\perp}$) can provide additional information regarding tissue microstructure and axon and myelin pathology (Counsell et al., 2006; Naismith et al., 2010; Song et al., 2003; Sun et al., 2006). DTI has been explored recently in the optic nerve examination of adult patients (Kolbe et al., 2009; Salmela et al., 2010; Trip et al., 2006; Wheeler-Kingshott et al., 2006; Xu et al., 2008), children (Filippi et al., 2012; Nickerson et al., 2010), monkeys (Coimbra et al., 2009), rodents (Song et al., 2003; Xu et al., 2008), demonstrating that DTI can be an effective means to evaluate the optic nerve abnormalities non-invasively.

Optic nerve development has been studied previously by using light and electron microscopic methods on specimens of humans (Dolman et al., 1980; Magoon and Robb, 1981), monkeys (Morrison et al., 1990; Sandell and Peters, 2001) and rats (Horsburgh and Sefton, 1986; Kuwabara, 1975). However, in vivo investigation of optic nerve is hindered by difficulties in accessing it non-invasively except for the intraocular part (optic nerve head). In recent years, DTI has been exploited increasingly to evaluate the optic nerve abnormality in adult and child patients. However, its application in

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the developmental examination of human and animal optic nerve after birth remains to be explored.

In comparison with the rodent models of optic nerve diseases, non-human primates (NHPs) resemble almost all aspects of human visual system including the visual pathway, functionality, and anatomical structure and provide an ideal model for investigating the developmental disorders of visual pathway, aging and neuropathology (Levkovitch-Verbin, 2004; Morrison et al., 1990; Sandell and Peters, 2001, 2002; Yang et al., 2009). The aim of the present study was to examine the diffusion property changes during the optic nerve development of NHPs from birth to adulthood by using DTI.

2. Materials and methods

2.1. Animals and preparations

Forty healthy rhesus monkeys aged from 2 weeks to 6 years (72 months) old were divided into 8 different age groups with 5 animals in each group (Table 1).

The animals were initially anesthetized with ketamine (5–10 mg/kg, IM), then orally intubated. An IV catheter was placed for delivering lactated ringers solution (3.5–10 ml/kg/h) in the scanner. Animals were anesthetized with 1–1.5% isoflurane mixed with 100% O_2 during scanning. The body temperature was maintained at 37.5 °C by a feedback-regulated circulating warm-water blanket. The anesthetized animals were breathing spontaneously and immobilized with a custom-made head holder and placed in the "supine" position in the scanner. Et-CO_2, inhaled CO_2, O_2 saturation, blood pressure, the mean arterial pressure (MAP), heart rate, respiration rate, and body temperature were monitored continuously and maintained in addition to visual inspection of animals every 30 min. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University in accordance with the NIH Guide for Care and Use of Laboratory Animals.

2.2. MRI

MRI scans were performed on a Siemens Trio 3 T scanner (Siemens Medical Solutions, PA, USA) with an 8-channel phase-array volume coil (INVIVO, Orlando, FL). A double-spin echo single-shot echo-planar imaging (EPI) sequence with GRAPPA (acceleration factor $R = 3$) was utilized with the acquisition parameters: TR = 5000 ms/TE = 86 ms, FOV = 83 mm × 83 mm, data matrix = 64 × 64, 1.3 mm isotropic resolution, b-value = 0, 1000 s/mm^2, 60 gradient directions, 10 averages. The total DTI acquisition time is about 60 min. High-resolution T1-weighted images were acquired with the 3D MPRAGE sequence for the structural identification of optic nerve. The acquisition parameters were: TR = 3000 ms, TE = 3.3 ms, TI = 950 ms, FOV = 116 mm × 116 mm, data matrix = 192 × 192, 0.6 mm isotropic resolution.

2.3. Data analysis

DTI data from each animal was motion- and eddy-current corrected and then averaged for generating MD, FA, $\lambda_\parallel$ and $\lambda_\perp$ maps by using FSL 4.1.3 (http://www.fmrib.ox.ac.uk/fsl/). Meanwhile, all the maps were interpolated by a factor of 2 in all the three dimensions. By using MRIcro 1.4 (http://www.mricro.com), regions of interests (ROIs) were selected manually on the coronal MD maps (Fig. 1(b), bottom image), and consisted of a set of consecutive pixels (~4 pixels in infants and ~7 pixels in adults) in the central optic nerve, across the slices located between the anterior and posterior quarters of each optic nerve (Fig. 1(b), top image). Also, the ROIs were cross-verified visually in the three-dimensional views of corresponding B0 images and FA maps. As no significant difference was observed between the left and right optic nerves in each age group (see Section 3 for more details), the values of MD, FA, $\lambda_\parallel$ and $\lambda_\perp$ in each ROI were calculated and averaged across both optic nerves of each animal for further analysis. The interpolation and mean value calculation were implemented with home-developed Matlab (The MathWorks, Inc.) scripts.

Numerical fitting of each DTI parameter was performed with the equation (Lebel et al., 2008):

$$\mathrm{MD\,(or\,FA},\lambda_\parallel,\lambda_\perp) = C + Ae^{-age/t}$$

where t is a time constant, indicating the time for a DTI parameter to reach 63.2% $(1-(1/e))$ of its amplitude parameter A and representing the evolutionary rate of the DTI parameter during optic nerve maturation. C is the maximal or minimal allowed value – the asymptotic value of MD, FA, $\lambda_\parallel$ or $\lambda_\perp$ in adulthood. In addition, the time to maturity is defined as the time to reach 90% of the amplitude A of a parameter, for comparison purpose. The curve fitting of each DTI parameter was carried out with OriginPro 8.5.0 SR1 (OriginLab Corporation, Northampton, MA, USA).

Paired T-tests were performed in each age group to compare the FA and diffusivity parameters between the left and right optic nerves to assure the dependence. One-way ANOVA followed by Tukey's post hoc test was carried out by using SPSS Statistics 17.0 (IBM Acquires SPSS Inc., Chicago, IL, USA).

3. Results

Paired T-test analysis showed that no significant difference in MD, FA, $\lambda_\parallel$ or $\lambda_\perp$ was seen between the left and right optic nerves in each age group (data not shown).

As illustrated in Fig. 2, the progressive changes in MD, FA, $\lambda_\parallel$, and $\lambda_\perp$ were well fitted into an exponential pattern. The time constants (t) for MD, FA, $\lambda_\parallel$ and $\lambda_\perp$, derived from the exponential curve fitting of each parameter, were 16, 14, 18 and 15 months after birth, respectively. The corresponding times to maturation for MD, FA, $\lambda_\parallel$ and $\lambda_\perp$ were 36, 33, 42 and 34 months (or 3, 2.8, 3.5, 2.8 years), respectively. At about 6 years (72 months) of age, MD, FA, $\lambda_\parallel$ and $\lambda_\perp$ reached 0.65×10^{-3} mm^2/s, 0.48, 1.07×10^{-3} mm^2/s and 0.44×10^{-3} mm^2/s, respectively.

The statistical results of MD, FA, $\lambda_\parallel$ and $\lambda_\perp$ changes over time are presented in Fig. 3. Significant changes in MD and FA, compared with younger age groups, were observed at 20 months of age and older, meanwhile, significant changes in $\lambda_\perp$ and $\lambda_\parallel$ were observed at 12 and 30 months of age and older, respectively. The significant differences between any two age groups are marked on the elder group (Fig. 3). FA increased by 87% from 2 weeks to 72 months, whereas MD, $\lambda_\parallel$ and $\lambda_\perp$ decreased by 35%, 17% and 49%, respectively.

Also, three monkeys from each age group were used for the inter-rater reliability assessment by performing the Bland-Altman analysis (by YY and SP). The coefficients of reproducibility for MD, FA, $\lambda_\parallel$ and $\lambda_\perp$ were 5.6%, 9.9%, 2.4% and 9.5%, respectively. The results indicated that the inter-rater differences of the DTI measures were generally within mean bias $\pm 2 \times$ standard deviation (data not shown), demonstrating excellent inter-rater reliability.

4. Discussion

Development of optic nerve has been investigated systemically on specimens of fetuses and postnatally developing albino rats by light microscopy (Kuwabara, 1975). Also, similar ex vivo examination has been performed in human and monkey optic nerves (Dolman et al., 1980; Magoon and Robb, 1981; Morrison et al., 1990; Sandell and Peters, 2001). To our knowledge, comparable in vivo examination of optic nerve development in either humans or animals after birth has not been reported.

As shown in Fig. 2, the progressive changes of FA and diffusivity parameters exhibited an exponential pattern during myelin maturation after birth, consistent with the DTI findings in human brain white matter in early life (Hermoye et al., 2006; Lebel et al., 2008). The results indicate that the optic nerve myelination has the similar developing pattern as seen in most brain white matter structures including corpus callosum, internal capsule, fornix, etc.

The time constants of MD, FA, $\lambda_\parallel$ and $\lambda_\perp$, which represents the growth rate during the optic nerve development, were 16, 14, 18 and 15 months respectively (Table 2). Accordingly, the times to maturation were 36, 33, 42 and 34 months, respectively, indicating these DTI parameters reached 90% of their maximum development in 2.8–3.5 years of age in macaque monkeys. Also, MD changes slightly lagged behind FA, consistent with that seen in the development of most human brain white matter (Lebel et al., 2008).

Generally, the aging in rhesus monkey proceeds about three times faster than in humans (Morrison et al., 1990; Tigges et al., 1988). Based upon this assumption, the time constants of the progression of MD and FA would be 3.9 and 3.6 human years. The converted time constant of FA in optic nerve is longer than those seen in some human brain white matter structures (Lebel et al., 2008), including inferior longitudinal fasciculus, splenium of the corpus callosum, genu and superior fronto-occipital fasciculus, but shorter than that in other white matter structures. On the other