Abnormalities in the Visual Pathway of Rats Subjected to Early Bilateral Enucleation Revealed by Diffusion Tensor Imaging

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Introduction Previous diffusion tensor imaging (DTI) studies have demonstrated congenital or early blindness is associated with significant atrophy and diffusion abnormalities in the optic white matter [1,2]. In contrast, such changes are absent in late, acquired blindness [3]. These results suggest that early deprivation of visual experience could have profound effects on neurodevelopment of the white matter tracts in the optic pathway. Bilateral enucleation is a widely-used animal model for studying neuroplasticity associated with blindness. It has been shown that early bilateral enucleation in rats can lead to structural and functional changes in the visual system, as well as in other parts of the brain [4,5]. In this study, DTI and high resolution rapid-acquisition relaxation-enhancement (RARE) imaging were used to investigate the morphological and structural changes in the brain of rats subjected to early bilateral enucleation.

Materials and methods *Enucleation surgery*: Sprague-Dawley rat pups were enucleated under 0.5% isoflurane anesthesia on postnatal day 4 (P4), when the ganglion cell axons had just entered the optic tract, and thalamocortical afferents had not yet arrived at the cortex [6]. The enucleated pups were revived by gentle warming under a 150 w lamp and returned to their mothers. Their litter mates were used as the control. All pups were separated from their mothers after weaning (P28), and housed in groups of 3-5 pups per cage. *MRI experiments*: All rats were imaged on a 7 T/20 cm Bruker Biospec scanner at approximately 4 months of age under 1.8-2.5% isoflurane anesthesia (in pure O_2). A volume coil was used for RF transmission, and a quadrature surface coil for signal detection. High-resolution anatomical images were acquired from 52 axial slices with a RARE sequence, FOV 3.5 cm×3.5 cm, matrix size 512×384, slice thickness 0.58 mm, TR 5800 ms, TE_{eff} 40 ms, RARE factor 4 and a total of 8 averages. DTI was performed with a spin-echo 4-shot EPI sequence, an encoding scheme of 30 gradient directions homogenously distributed on the unit sphere and the following acquisition parameters: TR 5000 ms, TE 26 ms, FOV 3 cm×3 cm, slice thickness 0.8 mm, matrix size 128×128, Δ 14 ms, δ 3 ms, b=0 and 800 s/mm^2 and 4 averages. *Data processing*: For each animal, the diffusion-weighted images were first realigned to the non-diffusion-weighted (b=0) image using the FMRIB's Diffusion Toolbox within FSL (http://www.fmrib.ox.ac.uk/fsl), and then interpolated into isotropic voxel size (117 μm×117 μm×117 μm). The elements in the diffusion tensor for each voxel were estimated by a multivariate linear fitting algorithm, and the tensor matrix was diagonalized to obtain its three pairs of eigenvalues and eigenvectors. Averaged fractional anisotropy (FA) and mean appear diffusion coefficient (ADC) were measured from the post-chiasmatic optic tracts (OT) and primary visual cortex (V1). Inter-group comparisons were performed with independent-sample *t* test.

Results Optic nerves (ON) and optic chiasm (OCH) were only barely visible on the RARE anatomical images acquired from the enucleated rats (Fig. 1). The post-chiasmatic OT appeared to be morphologically intact in the enucleated rats (Fig. 1), and had similar ADC, but significantly reduced FA, relative to those in the control rats (Fig. 2). No significant water diffusivity changes were observed in the V1 of the enucleated rats (Fig. 2).

Discussion The most interesting observation in this study is that bilateral enucleation at P4 had different effects on different parts of the visual pathway in rat. Profound atrophy was observed in the ON and OCH of the enucleated rats. This is consistent with the observation in human patients who underwent enucleation surgery at adulthood, showing significant shrinkage of the chiasm [1]. In rats subjected to monocular enucleation at the day birth, the ipsilateral optic nerve was almost totally absent [7]. The atrophy of the ON and OCH are likely the results of transneuronal degeneration induced by early deafferentation. The OT of the enucleated rats did not appear to be atrophic, but exhibited water diffusion abnormalities resembling those found in Wallerian degeneration (reduced FA and unchanged ADC). The diffusion abnormalities in the OT may be related to axonal disruption or loss, although demyelination and gliosis might have also contributed. The results suggest that bilateral enucleation at P4 may affect the development of post-chiasmatic optic tracts significantly. The V1 of the enucleated rats showed little changes in water diffusion, agreeing with the previous observations that the V1 in the subjects with early blindness is grossly normal [2].

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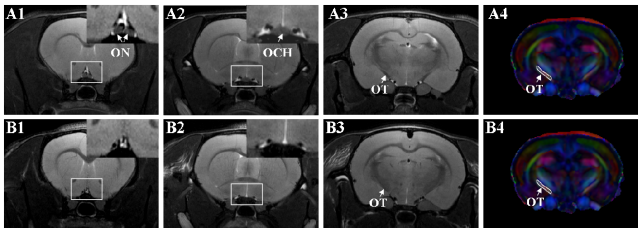

Figure 1. High-resolution RARE anatomical images (A1-A3 and B1-B3) and FA color maps (A4 and B4) acquired from a normal rat and a rat subjected to early bilateral enucleation at P4. ON: optic nerves, OCH: optic chiasm, OT: optic tracts.

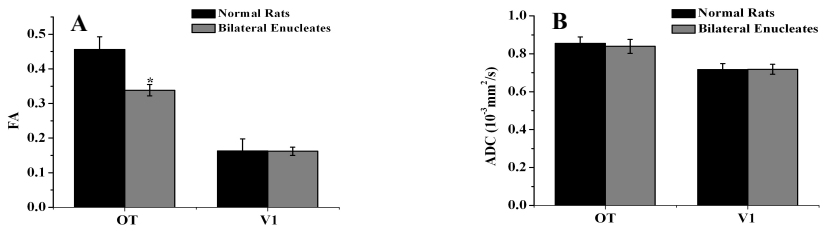

Figure 2. Average FA (A) and ADC (B) values in bilateral optic tracts (OT) and primary visual cortex (V1) of normal rats (n=9) and bilateral enucleates (n=9). *p<0.05, compared to the normal rats.