Bilateral Enucleation Before and After the Critical Period for the Specification of Interhemispheric Axonal Connectivity Induces Similar Changes on White Matter Fractional Anisotropy

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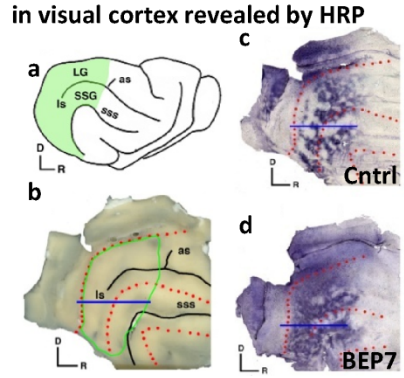
Introduction In several neurodevelopmental disorders, reduced water diffusion anisotropy (commonly reported as fractional anisotropy, FA) in white matter (WM) is associated with functional deficits in affected individuals. A potential interpretation of this observation is that reduced FA reflects an abnormal pattern of WM connectivity. To characterize the relationship between WM FA and perturbed axonal connectivity, we have compared diffusion tensor imaging (DTI) results between three groups of adult ferrets. Group 1 was bilaterally enucleated, as a form of visual deprivation, prior to the critical period during which retinal input is required for the normal specification of interhemispheric axonal connectivity in visual cortex, group 2 was bilaterally enucleated after the critical period for axonal connectivity specification, and group 3 consisted of normally-sighted ferrets.

Materials and Methods Four ferrets were enucleated on postnatal day (P)7 (BEP7 animals) and three were enucleated on P20 (BEP20 animals). Four normally-sighted control animals were also used in these studies. At maturity (P100 or later), multiple injections of the tracer horseradish peroxidase (HRP) were administered throughout visual cortex of one hemisphere for 3 animals from each group. After a survival period of 2 days, brains were perfusion fixed in 2% paraformaldehyde, and sectioned along the midline. Fractional anisotropy (FA) values were measured in postmortem tissue from hemispheres contralateral to HRP injections, using a high field (12 T) MRI scanner equipped with a small animal imaging gradient system, and specialized radio frequency circuitry built for these experiments, as described previously (1). Diffusion anisotropy in white matter was analyzed using the tract-based spatial statistics (TBSS) software package (http://www.fmrib.ox.ac.uk/fsl). Pair-wise t-statistics by all possible permutations were converted to p-values for each voxel in the WM skeleton created following the TBSS procedure. Subsequent to DTI, the patterns of retrogradely labeled somas and anterogradely labeled axon terminals in the contralateral hemisphere of HRP-injected animals were revealed in histological sections cut tangentially to the surface of the unfolded and flattened cortex (Fig. 1).

Results and Discussion Fig. 1a shows an illustration of an intact ferret brain, and Fig.1b shows an unfolded and flattened cortical mantle. Visual areas are shaded or outlined in green. Application of a threshold operation to the images of HRP-stained tissue (1) reveals that the percent of visual cortical area containing interhemispheric connections (dark-stained areas, Fig. 1c,d) is increased in BEP7 animals (59.2%) relative to control (49.7%). The cortical area difference from control animals is plotted in Fig. 2 (blue trace). TBSS analysis of FA parameter maps from BEP7 and control hemispheres demonstrated that FA is significantly ($p<0.05$) reduced for 1113 of 8125 WM skeleton voxels (Fig. 2, BEP7, red trace).

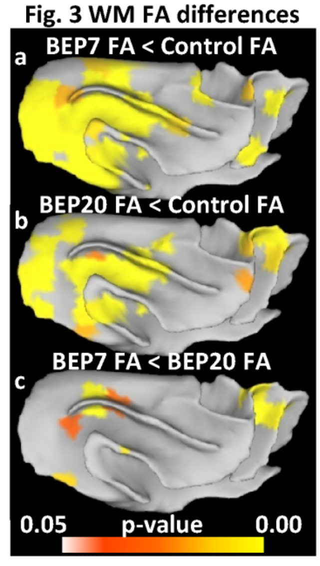
In contrast to BEP7 animals, the HRP staining pattern for BEP20 animals was similar to that of controls, with 50.2% of visual cortex exhibiting callosal connectivity (Fig. 2). The difference in callosal connnectivity between BEP7 and BEP20 animals indicates that the period during which retinal input is required for the development of normal pattern lies between P7 and P20. If the reduction in WM FA observed in BEP7 animals arose from the abnormal axonal connectivity pattern in early enucleated animals, normal FA would be expected in BEP20 animals. However, as shown in Fig. 2, 874 voxels in the WM skeleton exhibit reduced FA in BEP20 animals relative to control, indicating BEP20 WM is more similar to BEP7 WM than to control WM.

Fig. 1 Interhemispheric connectivity in visual cortex revealed by HRP

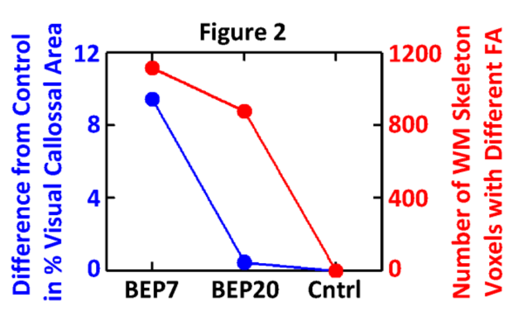


To assess the statistical significance of the DTI findings, a false discovery rate correction was performed to account for the large number of WM skeleton voxels. Each voxel was assigned to an 8×8×8 voxel cube, and for each cube, the binomial distribution was used to determine the probability that the observed number of voxels exhibit a p-value of less than 0.05. Cubes of less than 0.05 probability for comparisons between each group pair are projected onto surface models of the WM/cerebral cortical boundary in Fig. 3. As illustrated in Fig. 3a, FA differences between BEP7 and control animals are located within WM underlying visual cortical areas. Fig. 3b and 3c demonstrate that BEP20 animals exhibit closer similarity to the BEP7 group than to controls.

Fig. 3 WM FA differences



Conclusion Differences in WM FA between BEP20 and control animals are observed in spite of the fact that callosal axonal connectivity in BEP20 animals closely resembles that in normal ferrets. This finding suggests other factors, such as myelin and/or axonal structure, are the primary factors that determine the abnormal changes in WM FA that we observed in BEP7 and BEP20 animals.



Reference 1. Bock AS, et al., 2010. *Front. Syst. Neurosci*., 4:Article 149.