Injury to corpus callosum development with hypoxia: A behavioral DTI study on C57B/L6 mice

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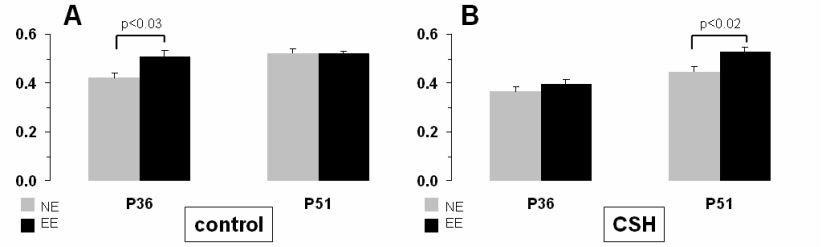
INTRODUCTION

Diffusion tensor imaging (DTI) of brain has gained wide acceptance as a tool for non-invasive microimaging of anatomical connectivity [1] and morphology [2]. The purpose here was to use *in vivo* DTI to assess potential effects of environmental enrichment during development, both under normal conditions and in a clinically-relevant neonatal rodent model of chronic sublethal hypoxia (CSH) injury to developing brain. This CSH model in rodents [3] mimics neuropathologic findings which accompany preterm birth infants (e.g., decreased gray, callosal and white matter volumes, cerebral ventriculomegaly, and behavioral deficits) [4,5]. Exposure of rodents to an enriched environment increases density and branching of the pyramidal cells [6], enhances neurogenesis, improves performance in several memory and learning tasks [7], and improves verbal and IQ test scores overt time in preterm very low-birth-weight infant [8]. Here we show the effects of enriched environment in changing local morphology during development, both in control and CSH mice.

MATERIALS and METHODS

Animal preparation: Four groups of C57B/L6 litters (P38 and P51), fostered by CD-1 dams, were reared under hypoxic (CSH, ambient O_2 = 10.2±1.0%) or normoxic (control; ambient O_2 = 22±1.0%) conditions from postnatal day 3 (P3). Both groups were reared under either non-enriched (NE) or environmentally enriched (EE) conditions from P11 to P35. The NE mice were reared under normal day-night cycles of the rodent care facilities, whereas the EE mice were housed in cages equipped with an activity wheel where the environment (i.e., wooden swing and various plastic and wooden toys like paper rolls, blocks, and rocks of different colors) was changed every 3 days. ***DTI:*** Mice were anesthetized with urethane (1 g/kg) and MRI experiments were performed on a 9.4T Bruker horizontal-bore system with custom-made surface coils [9]. DTI experiments were performed using a modified Stejskal-Tanner spin-echo diffusion-weighted sequence = 5 ms; Δ = 8 ms; TR/TE = 1000/18; NEX = 2; matrix = 128×128; FOV = 20×20 mm; slice thickness = 0.25 mm. Images were obtained with diffusion gradients applied in sixteen orientations with two diffusion sensitizing factors (approximately 0 and 1 ms/μm^2). Quantitative maps of fractional anisotropy (FA) were calculated and the primary eigenvectors were used to calculate directionally encoded color (DEC) maps to highlight the orientation of anisotropic tissues using medial-lateral (R for red), dorsal-ventral (G for green), and anterior-posterior (B for blue) color maps [10]. Since our prior study comparing developmental differences between normal and CSH mice implicated the corpus callosum [11] as a location of interest, this region was interrogated across these four groups (i.e., control NE, control EE, CSH NE, CSH EE).

RESULTS and DISCUSSION



In our prior study [11], we reported that the normal developmental changes in fiber organization within the corpus callosum were delayed by hypoxia. The most significant difference in FA between normal and CSH mice were observed at P45 and in particular these anisotropic changes were dominant in medial-lateral direction. At P38 the FA differences between normal and CSH mice did not exceed significance. In agreement with our prior findings, we demonstrate at P51 that FA in the corpus callosum is significantly different between control and CSH mice ($p<0.02$) and the anisotropy changes are also dominant in medial-lateral direction ($p<0.03$). Similarly in this study we also confirm our prior findings that at P36 the FA differences between normal and CSH mice are not very dissimilar. Upon behavioral input, however, there were some interesting FA changes observed within the corpus callosum in the same medial-lateral direction (A, B). In control mice, at P36 the EE group was significantly different from the NE group (A; $p<0.03$), whereas at P51 there were no significant differences between the two groups. In CSH mice, at P51 the EE group was significantly different from the NE group (B; $p<0.02$), whereas at P36 there were no significant differences between the two groups. In both control and CSH mice increases in FA may originate from the rise in the number of astrocytic processes in the corpus callosum [6]. Astrocytes are thought to promote myelin formation [12] and potassium channels expression, both of which may affect tissue anisotropy during maturation [13,14]. These results may have two implications for design of future studies: (i) environmentally enriched conditions can modify morphology, both in control and CSH mice; (ii) the delayed developmental changes in the corpus callosum in CSH mice can be partially circumvented by environmentally enriched conditions. These results may contribute to understanding of injury in preterm infants and the possible use of enriched environment for treatment.

REFERENCES

[1] Mori et al (1995) *MRM* 33 :41-52
[2] Zhang et al (2003) *NeuroImage.* 20 :1639-48
[3] Ment et al (1998) *Develop Brain Res.* 111:197-203
[4] Schwartz et al (2004) *Sem Perinatol.* 28:379-388
[5]Vaccarino & Ment (2004) *Arch Dis Child Fetal Neo.* 89:F190-192
[6] Dierssen et al (2003) *Cerebral Cortex.* 13:758-764
[7] Bruel-Jungerman et al (2005) *EJN.* 21:513–521
[8] Ment et al (2003) *JAMA*, 289:705-711
[9] Chahboune et al (2007) *NMR Biomed.* (in press)
[10] Pajevic and Pierpaoli (1999) *MRM.*42:526–540
[11] Chahboune et al (2006) *ISMRM*. 14:3398
[12] Soffie et al (1999) *Behav Brain Res*. 101:37-49
[13] Waxman et al (1989) *PNAS USA*. 86:1406-1410
[14] Vorisek et al (1997) *J Neurophysiol*. 78:912–919

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