Neurodevelopmental consequences of perinatal hypoxia in C57B/L6 mice assessed by in vivo DTI and behavior

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INTRODUCTION

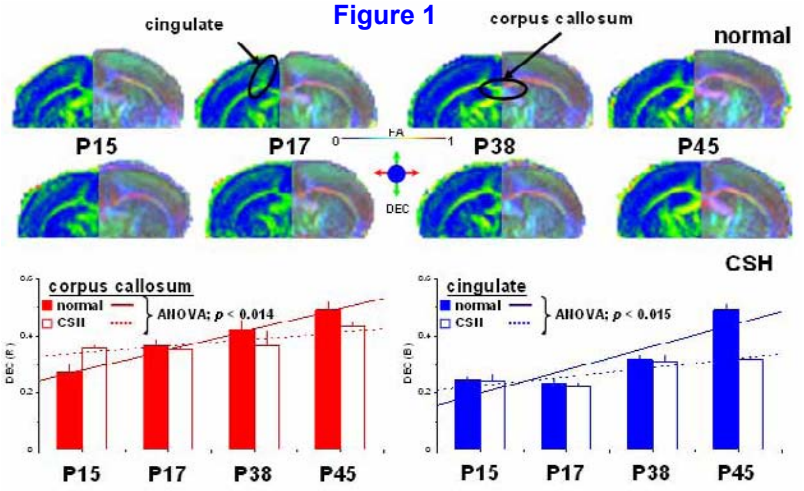
DTI is a superior anatomical imaging technique to probe tissue microstructure. The purpose here was to use DTI to assess potential alterations in morphology of developing axon pathways in a clinically-relevant neonatal rodent model of chronic sublethal hypoxia (CSH) injury to developing brain [1]. This CSH model in rodents is designed to replicate the prolonged cerebral hypoxemia which follows periventricular hemorrhagic infarction. The hypoxic injury mimics those neuropathologic findings which accompany preterm births (e.g., decreased gray, callosal and white matter volumes, cerebral ventriculomegaly, and behavioral deficits) [2,3] and results in altered maturation of the preterm brain and induces long-term changes in corticogenesis in the developing brain [4]. Here we show local modifications in brain development due to hypoxia, in agreement with histology and behavior, suggesting alterations in corticogenesis.

MATERIALS and METHODS

Animal preparation: C57B/L6 litters (P15 to P45), fostered by CD-1 dams, were reared under hypoxic (Normal; ambient O_2 = 9.5±1.0%) or normoxic (CSH; ambient O_2 = 22±1.0%) conditions from postnatal day 3 (P3). ***Behavior:*** Similarly reared groups of mice reared under either hypoxic or normoxic conditions from P3-P11 were exposed to normoxic oxygen levels from P12-P35 at which time they were behaviorally assessed. Spontaneous activity was assessed using an automated open field arena in 15-minute sessions over 3 consecutive days. Motor coordination and learning were also assessed using an accelerating Rotorod apparatus. ***DTI:*** The 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. 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. Diffusion weighting (b ≈ 1000 s/mm^2) in 15 different directions were acquired with one reference image (b ≈ 0 s/mm^2). Maps of fractional anisotropy (FA) were combined with maps of primary eigenvectors to calculate diffusion encoded color (DEC) maps [5]. The data were used to generate trajectory maps in dominant coordinates: medial-lateral, dorsal-ventral, and anterior-posterior. Five regions were examined: corpus callosum, caudet putamen, cingulate, forelimb cortex, and whisker barrel field.

RESULTS and DISCUSSION

Figure 1



Behavioral studies revealed significant levels of spontaneous activity for CSH mice than normal mice for total distance traveled and the velocity of movements during each 15 minute observation session. In contrast no differences were observed between the two groups on the Rotorod performance. These behavioral results suggest that CSH mice show no gross impairment in either the movement quality or simple motor learning as assessed by session improvement on the Rotorod task, but do reveal chronic increase in the baseline level of spontaneous activity. Whole brain *in vivo* DTI studies through development (P15 to P45) showed morphological changes in different brain regions (Fig. 1). The FA maps generally revealed a sharp contrast between tissue anisotropy changes (i.e., increase in white matter and decrease in gray matter) through maturation – earlier in age in normal than in CSH mice. There were no maturation-based changes in tissue anisotropy (i.e., FA or DEC values) in the forelimb and whisker barrel areas for either group (data not shown), which may partly support the behavioral results of no significant differences observed between the two groups on the Rotorod performance. The maturation-based changes in tissue anisotropy in the caudate putamen were similar in the normal or CSH mice (data not shown). However maturation-based changes in tissue anisotropy in the corpus callosum and cingulate were significantly altered by the hypoxic challenge early in development (Fig. 1). In particular the medial-lateral fibers (i.e., red in Fig. 1) in the corpus callosum and the anterior-posterior fibers (i.e., blue in Fig. 1) in the cingulate showed a progressive increase in tissue anisotropy through development in normal mice. The maturation of these fibers in these regions was significantly affected by hypoxia. The linear trends over normal development in both medial-lateral and anterior-posterior fibers in corpus callosum and cingulate, respectively, were significantly different from the trend over development in hypoxia. In conclusion, prior *in vitro* mouse brain DTI studies at high field [6,7] revealed tissue anisotropy variations in knockouts (e.g., Bcl-x knock-out or myelin-deficient shiverer mutant). Our results here demonstrate that *in vivo* DTI at high magnetic field can detect normal developmental changes and differentiate changes in development as a consequence of hypoxia, which is a moderate perturbation in comparison to genetic manipulations. The changes in morphology in the corpus callosum and cingulate are generally consistent with histology as well as behavioral changes, demonstrating alterations in corticogenesis with hypoxia. These *in vivo* DTI results in the CSH model are important for understanding neurodevelopmental difficulties that are found in low birth weight preterm infants.

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