Effects of environment enrichment on hypoxia-induced injury to corpus callosum and cingulate in C57B/L6 mice

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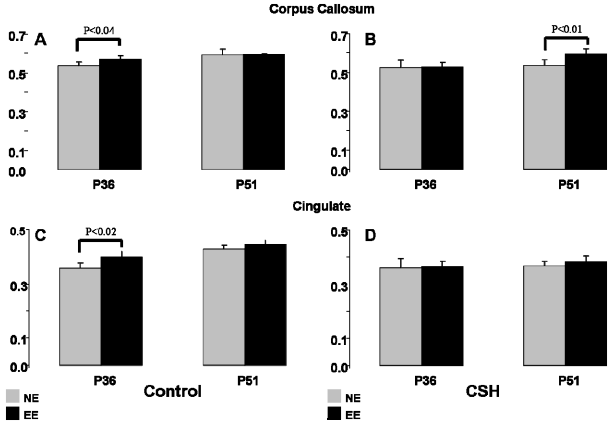
INTRODUCTION

Brain DTI is a widely used tool for the noninvasive detection of changes in microstructural organization during normal development [1-3] and as a result of pathological conditions such as cerebral plasy [4], and diffuse brain injury [5]. Here we used *in vivo* DTI to assess the possible effects of early environmental enrichment on the developing brain under normal conditions and in a neonatal rodent model of chronic sublethal hypoxia (CSH) injury. The CSH rodent model [6] mimics many of the neuropathologic findings which accompany preterm birth in human infants and leads to an altered pattern of maturation for the corpus callosum and cingulum when viewed using DTI. Exposing rodents to an enriched environment increases the density and branching of pyramidal cells [7], enhances neurogenesis, and improves performance on memory and learning [8] tasks. Similarly, preterm infants exposed to an enriched environment evidence improved IQ scores over time [9].

MATERIALS and METHODS

Animal preparation: Four groups of C57B/L6 litters (P36, P51), fostered by CD-1 dams, were reared under normoxic (control; ambient O_2 = 22±1%) or hypoxic (CSH, ambient O_2 = 10±1%) conditions from P3. Both groups were reared with either non-enriched (NE) or environmentally enriched (EE) conditions from P11 to P35. The NE mice were reared under normal vivarium cage conditions, whereas the EE mice were housed in larger cages equipped with an activity wheel and a variety of toys that where changed every 3 days. ***DTI:*** Mice were anesthetized with urethane (1 g/kg). DTI experiments were performed on a 9.4T Bruker horizontal-bore system [2] 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 (0, 1 ms/μm^2). Maps of fractional anisotropy (FA) were calculated and primary eigenvectors were used to calculate directionally encoded color (DEC) maps to highlight the orientation of anisotropy using medial-lateral (red), dorsal-ventral (green), and anterior-posterior (blue) color maps [10]. Our prior studies comparing developmental differences between normal and CSH mice indicated that the corpus callosum and cingulate [11] have significant alterations in the pattern of maturation. As a result, in the present study we examined these same regions in control NE and EE mice and in CSH NE and EE mice. We also examined the performance of these four groups on spatial memory using the Morris Water Maze at 4-5 months of age.

RESULTS and DISCUSSION



Previously, [11] we reported that normal developmental changes in fiber organization within the corpus callosum and cingulate are delayed by early postnatal hypoxia. The most significant difference in FA between normal and hypoxic mice was observed between P45 and P51. The DEC data showed that the anisotropic changes were dominant in medial-lateral and anterior-posterior directions, respectively, in the corpus callosum and cingulate. No FA differences were found at P38 or earlier. Consistent with our previous findings, in the present study we observed significantly different FA values in the corpus callosum and cingulate between control and CSH mice at P51 and the anisotropy changes remained dominant in the medial-lateral ($p<0.03$) and the anterior-posterior directions ($p<0.006$). In addition, we also confirmed our prior findings that FA values at P36 were not significantly different between normal and CSH mice. In contrast, exposure to environmental enrichment from P11-P35 significantly altered the organization and maturation of FA changes in the corpus callosum (A,B) and cingulate (C,D). In control mice, examined at P36 the EE group was significantly different from the NE group in both regions, whereas at P51 there were no significant differences between the two groups in either region. At P51, CSH EE mice were significantly different from the NE group in corpus callosal FA (B; $p<0.01$), whereas no significant differences were found in either region at P36. Behavioral comparison of these groups of mice on probe trials after 8 days of training in a Morris Water Maze revealed hypoxic mice spend significantly less time in the quadrant in which the submerged platform was present during the training trials ($p=0.02$) and have shorter latencies to enter the training quadrant ($p=0.04$). Hypoxic animals exposed to EE spent significantly more time in the training quadrant than did Hypoxic mice (47% versus 32%, $p=0.007$) and were not significantly different from Normoxic mice on this measure (47% versus 41%). 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 suggest that environmental enrichment can modify morphology and affect the delayed developmental changes with hypoxia. These results may contribute to understanding injury in preterm infants and possible use of enriched environment treatment.

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