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Alterations of hippocampal projections in adult macaques with neonatal hippocampal lesions: A Diffusion Tensor Imaging study

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ABSTRACT

Neuropsychological and brain imaging studies have demonstrated persistent deficits in memory functions and structural changes after neonatal neurotoxic hippocampal lesion in monkeys. However, the relevant microstructural changes in the white matter of affected brain regions following this early insult remain unknown. This study assessed white matter integrity in the main hippocampal projections of adult macaque monkeys with neonatal hippocampal lesions, using diffusion tensor imaging (DTI). Data analysis was performed using tract-based spatial statistics (TBSS) and compared with volume of interest statistics. Alterations of fractional anisotropy (FA) and diffusivity indices were observed in fornix, temporal stem, ventromedial prefrontal cortex and optical radiations. To further validate the lesion effects on the prefrontal cortex, probabilistic diffusion tractography was used to examine the integrity of the fiber connections between hippocampus and ventromedial prefrontal cortex, and alterations were found in these connections. In addition, increased radial diffusivity in the left ventromedial prefrontal cortex correlated negatively with the severity of deficits in working memory in the same monkeys. The findings revealed microstructural changes due to neonatal hippocampal lesion, and confirmed that neonatal neurotoxic hippocampal lesions resulted in significant and enduring functional alterations in the hippocampal projection system.

Introduction

The hippocampus exhibits a pronounced vulnerability to hypoxic, ischemic or metabolic noxious events and behavioral stress, and is highly susceptible to epileptogenic mechanisms that have been associated with a spectrum of neurological diseases and psychiatric disorders (Bartsch, 2012). For example, children and adolescents that had suffered hypoxia-ischemia perinatally or later in childhood due to cardiac, respiratory, or other neurological disorders or their treatments have been diagnosed with severe long-term memory impairment (Adlam et al., 2009; de Haan et al., 2006; Gadian et al., 2000; Vargha-Khadem et al., 1997). Memory impairment in these cases has been mainly ascribed to selective bilateral hippocampal atrophy (Bachevalier and Vargha-Khadem, 2005). However, alterations of other brain structures, such as posterior thalamus, putamen, and retrosplenial cortex are also present in these cases (Vargha-Khadem et al., 2003) and could likewise contribute to the memory impairment. These additional neural changes could have resulted from direct impact of the hypoxia-ischemia insult and treatments or to plastic changes following early hippocampal atrophy. While this question remains unresolved in the clinical literature, experimental lesion studies in nonhuman primates could provide important clues on the source of the widespread cognitive and neural impacts of neonatal hippocampal damage.

Like the memory impairment reported in children with perinatal hippocampal atrophy, recent reports on the effects of neonatal hippocampal lesions in nonhuman primates (NHPs) have indicated severe and long-lasting deficits in relational memory processes known to be mediated by the hippocampus (Eichenbaum, 2003; O'Keefe and Nadel, 1978; Squire et al., 2007). These deficits include impairment in object recognition memory (Zeamer and Bachevalier, 2013; Zeamer et al., 2010), memory for food/place associations (Glavis-Bloom et al., 2013), and memory for spatial locations and object/place associations (Blue et al., 2013). Interestingly, the neonatal hippocampal lesions appeared

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to have altered widespread neural systems given that the same animals also presented with loss of working memory processes (Heuer and Bachevalier, 2011a, 2013) thought to be mediated by the lateral prefrontal cortex (Curtis and D'Esposito, 2004; Owen, 2000; Petrides, 2005). Direct evidence of such large scale neural reorganization after early hippocampal lesions in the monkeys has not been fully explored. Earlier studies demonstrated significant changes in the maturation of prefrontal neurons after neonatal medial temporal lobe lesions (Bertolino et al., 1997; Chlan-Fourney et al., 2000, 2003). However, these neural alterations could not be entirely associated with damage to the hippocampus since the neonatal lesions were large, including not only the hippocampus but also the amygdala and adjacent cortical areas. Therefore, the impact of selective neonatal hippocampal lesions on brain functions and microstructure in monkeys remains to be fully investigated. As a first step towards investigating the long range neural changes that may have resulted from early damage to the hippocampus, the present study assessed the integrity of the hippocampal projections in monkeys that had received bilateral damage to the hippocampus in the first week of life using Diffusion Tensor Imaging (DTI).

DTI is a valuable tool for investigating microstructural integrity and connectivity of neuronal fibers non-invasively, providing a possible biomarker of disease progression and a robust approach to investigate the neuronal substrates for abnormal behaviors of animals and humans. Several useful measures based on DTI have found to be sensitive to a series of white matter-related disruptions in the brain (Le Bihan et al., 2001). For example, fractional anisotropy (FA), a scalar measure of the degree of anisotropic water diffusion of brain tissues, and mean diffusivity (MD) that characterizes the overall displacement of water molecules, are thought to relate to the microstructural features of white-matter organization (Beaulieu, 2002). In addition, recent studies have shown that the three eigenvalues of the diffusion tensor matrix can be further separated into components parallel and perpendicular to local axon tracts, with the former defined as axial diffusivity (D_a) and the average of the latter two defined as radial diffusivity (D_r). D_a and D_r are suggested to indicate different patterns of underlying pathological alterations, such as the disruption and loss of axonal membranes and myelin in the fiber tracts in the brain and also the alterations in the size, density and organization of axons (Le Bihan, 2003; Song et al., 2002, 2003). Thus, the patterns of directional diffusivity alteration may be useful in further revealing the nature of white matter disruptions resulting from neonatal hippocampal lesions, and could provide more sensitive measures to explain behavioral and cognitive changes reported after such early lesions.

DTI has been applied to evaluate changes in the white matter microstructure in diseases related to hippocampal damage in human and NHP models (Kubicki et al., 2007; Lee et al., 2012; Shamy et al., 2010). In particular, abnormal FA and diffusivity changes were observed in the fornix and ventromedial prefrontal cortex of macaque monkeys with hippocampal lesions received in adulthood (Shamy et al., 2010). This report suggested that hippocampal damage acquired in adulthood results in altered connections between the hippocampus and cortical regions through the fornix. Given the greater reorganization of brain connectivity usually found after early-onset versus adult-onset brain insults (Kolb et al., 2010; Payne and Cornwell, 1994), it is likely that alterations in the connectional networks of the hippocampus may also be present, or even more pronounced, after early-onset lesions. To test this hypothesis, we performed several processing analyses of DTI data obtained on animals with neonatal hippocampal lesions and their controls, in which detailed behavioral and cognitive characterization was obtained throughout development. Tract-based spatial statistics (TBSS) performs medium-resolution nonlinear registration followed by projection onto an alignment-invariant tract representation, which is a robust and sensitive approach for voxelwise multiple-subject comparisons of DTI data (Smith et al., 2006). This analysis was used to evaluate the integrity of the main hippocampal projection of adult monkeys with neonatal hippocampal lesions and their age-matched controls. To compare and further validate the results, an evaluation of white matter changes was performed on the areas of the hippocampal projections by volume of interest analysis, and on the delineated fiber tracts connecting hippocampus and ventromedial prefrontal cortex by probabilistic diffusion tractography performed on the DTI data with high angular resolutions (Behrens et al., 2003, 2007). Finally, to examine whether the enduring recognition and working memory deficits after neonatal hippocampal lesions were related to changes in areas onto which the hippocampus projects, correlation analysis was performed between recognition and working memory scores and the DTI-derived measures.

Methods and materials

Animals

Ten adult rhesus macaques (*Macaca mulatta*) of both sexes and aged 8 to 10 years were utilized. Five had received neurotoxic lesions of the hippocampus (Group Neo-Hibo: 3 males, 2 females), induced with bilateral injections of ibotenic acid (5.0 μl) at the age of 10–12 days, and five age-matched controls had received sham lesions (Group Neo-C: 2 males, 3 females). Details of the MRI-guided surgical procedures, lesion evaluation and rearing conditions were reported previously (Goursaud and Bachevalier, 2007; Zeamer et al., 2010). Development of cognitive functions was evaluated in the same cohorts of animals at different time points during development and included measures of object recognition memory (Heuer and Bachevalier, 2011a; Zeamer and Bachevalier, 2013; Zeamer et al., 2010), memory for location and object/place associations (Blue et al., 2013), memory for food/place associations (Glavis-Bloom et al., 2013) and working memory (Heuer and Bachevalier, 2011b, 2013).

Neuroimaging procedures

All procedures were approved and applied in full compliance with the Institutional Animal Care and Use Committees (IACUC) of Emory University, and were in line with the policies outlined in the NRC Guide for the Care and Use of Laboratory Animals (2011, 8th ed).

For the scanning procedures, animals were anesthetized with 1–1.5% isoflurane mixed with 100% O_2 and immobilized in a supine position in a custom-made head holder. Et-CO_2, inhaled CO_2, O_2 saturation, blood pressure, heart rate, respiration rate, and body temperature were monitored continuously and body temperature was maintained with a warm blanket surrounding the animal as described previously (Li et al., 2013). An intravenous drip of 0.45% dextrose and sodium chloride was administrated to ensure normal hydration.

All MRI experiments were performed on a Siemens 3T Trio scanner (Siemens Medical Solutions USA, Inc., Malvern, PA). Diffusion images were acquired with a Siemens 8-channel phased-array volume coil and a dual spin-echo, echo planar imaging (EPI) sequence with GRAPPA (R = 3) and the following imaging parameters: TE = 96 ms, TR = 5700 ms, FOV = 96 mm × 96 mm, data matrix = 74 × 74, voxel size = 1.3 mm × 1.3 mm × 1.3 mm. DTI data were collected at a single b-value of 1000 s/mm^2 with 60 diffusion encoding directions chosen to be approximately isotropically distributed on a sphere according to the electrostatic repulsion model (Jones et al., 1999). We acquired 5 repetitions of DTI data sets with the phase-encoding direction in the anterior–posterior (A–P) axis and another 5 repetitions with identical imaging parameters except for reversed phase-encoding direction (P–A) for correcting susceptibility-related distortion with TOPUP function in FSL (Andersson et al., 2003). Each 5 repetitions with the identical phase-encoding direction was co-registered using rigid-body affine transformation and then averaged to improve the signal to noise ratio (SNR). T_1-weighted images were acquired by using a 3D MPRAGE sequence with GRAPPA (R = 2) with the following parameters: inversion time = 950 ms, TE/TR = 3.5 ms/3000 ms, FOV = 96 mm × 96 mm, matrix = 192 × 192, 6 averages, and were used for structural