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Diffusion Tensor Imaging Reveals Microstructural Alterations in Corpus Callosum and Associated Transcallosal Fiber Tracts in Adult Macaques with Neonatal Hippocampal Lesions

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Abstract

Neonatal hippocampal lesions can result in long-term effects on the morphological and functional integrity of the adult brain. To investigate the effects of neonatal hippocampal lesions on the microstructural integrity of corpus callosum in adulthood, macaque monkeys ($n = 5$) received neonatal bilateral hippocampal lesion (Neo-Hibo) induced by infusion of ibotenic acid at 1-2 weeks of age and were scanned using diffusion tensor imaging (DTI) at 8-10 years old. Age and gender -matched control animals that had received sham operation (Neo-C, $n = 5$) at 1-2 weeks of age were scanned for comparison purpose. Corpus callosum was segmented into seven regions that were grouped into anterior corpus callosum (rostrum, genu, rostral body and anterior midbody), posterior corpus callosum (posterior midbody, isthmus and splenium) for data analysis. The associated transcallosal fiber tracts were delineated by using probabilistic tractography and evaluated with TBSS. Significantly increased diffusivity indices (mean, axial and radial diffusivity) were observed in the posterior segments of corpus callosum. Also, significant decreased fractional anisotropy (FA) and increased diffusivity indices were seen in the associated transcallosal fiber tracts proximal to motor, posterior parietal and retrosplenial cortices. Increased mean diffusivity (MD) in posterior midbody negatively correlated with reduction of surface area of corpus callosum in Neo-Hibo monkeys. Also, the magnitude of the memory impairments was significantly correlated with FA in transcallosal fiber tracts across splenium in Neo-Hibo animals. Although no microstructural changes were observed in the anterior segments of corpus callosum, changes in FA values and diffusivity indices were observed in the white matter fibers of the ventromedial prefrontal cortex. This DTI study revealed that neonatal hippocampal lesion resulted

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in enduring degradation in the adult transcallosal fibers proximal to parietal and retrosplenial cortices, and hemispheric connections through posterior corpus callosum. The findings may provide complementary information for understanding the neural substrate of behavioral and cognitive deficits observed in patients with early insult to the hippocampus.

Keywords

DTI; TBSS; hippocampus; corpus callosum; non-human primate

Introduction

Adult macaque monkeys with selective neonatal hippocampal lesions provide a unique primate model to evaluate the long-term effects of neonatal hippocampal lesion on the morphological and functional integrity of the adult brain (Machado et al., 2008; Zeamer et al., 2010). Using diffusion tensor imaging (DTI), we have demonstrated significant microstructural changes in the hippocampal projection system (fornix, temporal stem, ventromedial prefrontal cortex and optic radiations) in adult monkeys that received neonatal hippocampal lesions (Meng et al., 2014). In addition, a resting-state functional MRI study in the same animals revealed significant differences in the patterns of dorsolateral prefrontal functional networks with altered interactions of core regions of the working memory network (medial prefrontal and the posterior parietal cortices) as well as cortical regions within the object perception and motion pathways in the temporal lobes (Meng et al., 2016). Finally, hypometabolism in the retrosplenial cortex was seen in monkeys after neonatal hippocampal lesions in a PET study (Machado et al., 2008), suggesting altered functional connectivity between the hippocampus and the retrosplenial cortex via the posterior corpus callosum (CC).

The posterior portion of CC (posterior midbody, isthmus and splenium), contains fibers projecting from the posterior parietal cortex to the hippocampus via the parahippocampal and entorhinal areas and those from the hippocampus to the retrosplenial cortex via the presubicular cortex (Duvernoy, 2005; Jones and Peters, 1987; Kobayashi and Amaral, 2003; Kobayashi and Amaral, 2007). Thus, we recently explored the impact of the neonatal hippocampal lesions on the maturation of CC by measuring the surface areas of different segments of CC using structural MRI when the same animals were 1.5 years of age (Payne et al., 2017). Although the total CC surface area in animals with neonatal hippocampal lesions did not differ from control animals, reliable increases were observed in the rostrum and the isthmus, suggesting altered inter-hemispheric fibers from the ventromedial prefrontal cortex, posterior parietal, and retrosplenial regions, which traverse the rostrum and isthmus subregions of the CC, respectively (Schmahmann and Pandya, 2006; Witelson, 1989). Therefore, we hypothesize that the integrity of the posterior CC microstructure in matured brains may be impaired due to neonatal hippocampal lesions.

Diffusion tensor imaging (DTI) has been widely applied to quantitatively identify the microstructural integrity of brain white matter fiber bundles. Fractional anisotropy (FA), a scalar measure of the degree of anisotropic water diffusion of brain tissues, and mean

diffusivity (MD), axial and radial diffusivities (Da and Dr) that characterize the displacement of water molecules in the tissue, are useful to evaluate the axon integrity including axonal membranes and myelin in the fiber tracts of the brain (Le Bihan et al., 2001). Probabilistic tractography with DTI data can produce a likelihood map of the diffusion paths of the fiber tracts through regions of interest (ROIs) (Behrens et al., 2003). Prior human DTI tractography results demonstrated CC could be parcellated into six different subdivisions based on trajectories to different cortical regions (Huang et al., 2005). In the present study, DTI probabilistic tractography was employed to delineate the associated transcallosal fiber tracts across CC, and tract-based spatial statistics (TBSS), which performs high-dimensional nonlinear registration, was used to evaluate the potential changes in the CC and its associated transcallosal fiber tracts (Smith et al., 2006).

Methods

Animals

Rhesus monkeys received neurotoxic lesions of the hippocampus (Group Neo-Hibo, $n = 5$) induced by infusion of ibotenic acid (5.0 μ l) bilaterally, at the age of 10-12 days after birth. Another group of age-matched animals received sham lesions and were used as controls (Group Neo-C, $n = 5$). Details of the surgical procedures as well as of extent of hippocampal lesions for the same animals were previously reported (Courtney and Jocelyne, 2017; Goursaud and Bachevalier, 2007; Zeamer et al., 2010). These animals were scanned with MRI at the age of 8-10 years.

During MRI scanning, the animals were anesthetized with 1.0-1.5% isoflurane mixed with 100% O₂ and immobilized with a custom-made head holder in the MRI scanner. Physiological parameters, such as Et-CO₂, inhaled CO₂, O₂ saturation, blood pressure, heart rate, respiration rate, were monitored continuously and maintained in normal ranges. Rectal temperature was maintained at a normal range with a warm blanket surrounding the animal. An intravenous drip of 0.45% dextrose and sodium chloride was placed to prevent the animals from dehydration.

All procedures were approved and used in full compliance with the Institutional Animal Care and Use Committees of Emory University and were in line with the policies outlined in the NIH Guide for the care and use of laboratory animals.

MRI Procedures

All MRI procedures were performed on a Siemens 3T Trio scanner (Siemens Medical Solutions, Inc., PA). DTI images were acquired with an 8-channel phase-array volume coil and a dual spin-echo, phase-reversal echo planar imaging (EPI) MRI sequence with GRAPPA (R=3) and the following imaging parameters: TE = 96 ms, TR = 5.7 s, FOV = 96 × 96 mm, data matrix = 74 × 74, 5 averages, voxel size = 1.3 mm × 1.3 mm × 1.3 mm, 60 gradient directions with b values = 0, 1000 s/mm². Five repetitions were applied 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 subset of 5 repetitions with the identical phase-encoding direction was co-registered using rigid-body affine transformation and then averaged in order to improve the signal to noise ratio (SNR). Prior to DTI data analysis, a macaque brain template was built from the T1-weighted images acquired on the same animals by using the 3D MPRage sequence with GRAPPA (R=2) and the following parameters: inversion time = 0.95 s, TE / TR = 3.5 ms / 3 s, FOV = 96 mm × 96 mm, data matrix = 192 × 192, 6 averages.

Data Analysis

DTI data were processed with the FSL package (FMRIB, Oxford) and MATLAB (Mathworks, Natick, MA) scripts off-line. Pre-data processing, including eddy-current image distortion correction and co-registration, was applied prior to image analysis. Susceptibility-induced DTI image distortion correction was performed by the phase-reversal approach (Andersson et al., 2003). By using TBSS toolbox in FSL, FA, MD, Da and Dr maps were nonlinearly registered to the population-specific FA template built from all the control animals, and then skeletonised by searching the surface perpendicular to the local skeleton structure for the maximal values to produce white matter fiber tracts and minimize the partial volume effect (Smith et al., 2006). The CC was divided into 7 segments including rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium, as defined by Witelson (Witelson, 1989). Probabilistic tractography was used to produce a likelihood map of the diffusion paths across the CC segments. The derived fiber tracts were normalized by the total numbers of fibers in the tracts with 0.2% threshold and averaged across control animals and then binarized. After confirming normal distribution of the data by a one-sample Kolmogorov-Smirnov test, standard two sample t-tests was used to voxelwisely test the difference within the skeletonized maps of the DTI-derived CC measures and associated transcallosal fiber tracts, with false discovery rate (FDR) multiple comparison correction with q value = 0.05.

To investigate whether the changes of the DTI-derived measures were related to the morphological changes in the corresponding CC segments of Neo-Hibo animals, the surface area of each segment (i.e., rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium) was calculated respectively based on the T₁-weighted images. Pearson's correlation analysis was used to test the relationship between the reduced surface areas and the averaged DTI-derived measures in the CC segments with significant group difference of P-value less than 0.05 considered statistically significant.

Correlations with behavioral performance in Neo-Hibo animals

Visual Paired Comparison (VPC) tasks were used to assess the visual and spatial recognition memory mediated by the medial temporal lobe (Bachevalier and Nemanic, 2008; Nemanic et al., 2002), and the object self-ordered (Obj-SO) and serial-order memory (SOMT) tasks were used to assess working memory mediated by the dorsolateral prefrontal cortex (Petrides, 1991; Petrides, 1995a; Petrides, 1995b). The recognition and working memory performance included: 1) the total errors to reach learning criterion in self-order working memory task obtained (Heuer and Bachevalier, 2011b), 2) the percent looking at novel pictures at delays of 120 s in visual paired comparison (VPC-delay) task at 4 years of age (Zeamer and Bachevalier, 2013), and 3) the percent looking at the novel re-arranged images

in the VPC-object-in-place (VPC-spatial) at 5-6 years of age (Blue et al., 2013). Pearson's correlation analyses were conducted to determine correlations between memory scores and the DTI indices with significant group differences.

Results

Coronal T1-weighted images through the anterior portion of the medial temporal lobe (Fig. 1) illustrated the extent of bilateral hippocampal lesions (asterisks) and sparing (white arrows) of entorhinal (Brodmann area 28) and perirhinal (Brodmann areas 35/36) cortices in one representative case with neurotoxic hippocampal lesions (Neo-Hibo) bilaterally and one sham-operated control (Neo-C).

As shown in Figure 2, no significant FA changes were observed in the posterior CC. Yet, increased diffusivities were detected in the posterior midbody (MD, Da and Dr), the isthmus (MD and Da), and the splenium (Dr) (see Fig. 2a). In coronal views, decreased FA and increased diffusivities (MD, Da and Dr) extended to the lateral callosal fibers proximal to the posterior parietal cortex in the transcallosal fiber tracts across isthmus (Fig. 2d and e), and the motor cortex in the transcallosal fiber tracts across posterior midbody (Fig. 2c) and isthmus (Fig. 2d). Decreased FA and increased diffusivities (MD, Da and Dr) were seen proximal to the retrosplenial cortical areas (Fig. 1f). Although no FA or diffusivities changes were observed in the anterior corpus callosum (rostrum, genu, rostral body, and anterior midbody), significant changes of these DTI indices were observed in the medial prefrontal cortical area in the transcallosal fiber tracts across rostrum or genu (Fig. 2b).

As illustrated in Table 1, the reduced CC surface area in Neo-Hibo animals was seen in posterior midbody ($p = 0.05$), but not in the other segments ($p = 0.28$). In the posterior midbody, the reduced surface areas in Neo-Hibo animals negatively correlated with increased MD ($p < 0.05$, Figure 3), whereas the diffusivities in other posterior CC had insignificant correlations with the CC thickness and $r < 0$ (Table 2).

As illustrated in Table 3, the memory scores in VPC-delay task were significantly correlated with FA in transcallosal fiber tracts across splenium in Neo-Hibo animals, but not with any DTI-measures in other areas.

Discussions

This DTI study provides the first demonstration of significant microstructural changes in matured corpus callosum fiber bundles due to neonatal hippocampal injury using a NHP model. These changes were evident mainly in the posterior segments of the corpus callosum, although the white matter alterations were also seen more anteriorly in transcallosal fibers of the medial prefrontal cortex. These changes in DTI indices corroborate and extend prior findings indicating alterations of the surface areas of the isthmus and the rostrum of the CC when the animals were scanned at the juvenile age (Payne et al., 2017). As discussed below, these morphological and/or microstructural changes in CC and associated transcallosal fibers suggest neonatal hippocampal damage could result in a widespread impact to cortical areas anatomically and functionally connected with the hippocampus.

White Matter Changes in the Corpus Callosum

Our DTI results indicate that early bilateral hippocampal lesions caused more profound alterations of the microstructural integrity in the posterior than the anterior corpus callosum. As compared to controls, animals with Neo-Hibo showed reduction in the surface area of the posterior CC. The surface areas reduction was also associated with decreased FA and increased mean diffusivity in posterior transcallosal fibers proximal to posterior parietal and retrosplenial cortices (see Table 1 and Figure 2). Interestingly, the increase in MD values in the posterior midbody correlated with the reduced surface areas of this CC segment in animals with Neo-Hibo lesions. These findings are consistent with those of a previous report of hypometabolism in the retrosplenial cortex of 4-5 years old adult macaques with neonatal hippocampal lesion examined with positron emission tomography (PET) (Machado et al., 2008).

Although the white matter changes may be ascribed to the neonatal hippocampal damage, alternative explanations need to be discussed. First, the macaque monkey hippocampus has minimal callosal connections and most of the callosal fibers emanating from the hippocampal system come from the entorhinal, perirhinal and parahippocampal regions - not the hippocampus per se (Duvernoy, 2005; Jones and Peters, 1987; Kobayashi and Amaral, 2003; Kobayashi and Amaral, 2007), it is thus possible that the white matter changes observed in the posterior CC segments may have resulted from damage to these medial temporal cortical areas. Yet, as described in an earlier report describing the extent of lesions for all of the five Neo-H used in the current study (Zeamer et al., 2010), damage outside the hippocampus included only small portion of areas TH/TF (mean: 6.5%) on the parahippocampal gyrus and the posterior amygdala (2.5%); no damage was identified for the entorhinal and perirhinal cortices. Thus, it is unlikely that this minimal extradamage resulted in the white matter microstructural changes in the posterior CC. However, given the extensive connections between the hippocampus and these medial temporal cortical areas, damage to the hippocampus may have affected the structure and function of these cortical areas, especially when the hippocampal insult occurred early in development. Therefore, the changes in white matter could have resulted from an indirect impact of neonatal hippocampal damage on the medial temporal cortical areas. In line with this proposal, we have already shown decreased functional connectivity in dorsolateral prefrontal cortical networks in the same Neo-H animals, including functional connectivity between parietal cortex and medial temporal cortical areas (Meng et al., 2016). Hence, the Neo-H lesions have a widespread impact on structures not necessarily receiving direct projections from the hippocampus.

A second alternatively explanation may relate to potential direct damage to the dorsal cortical areas during the lowering of the needle during the ibotenic acid lesions. We re-examined the FLAIR images taken on the five Neo-H animals taken a week post-surgery to identify potential hypersignals in the cortical areas where the injection needles were lowered. None of the cases presented with hypersignals in the cortical areas or in the vicinity of the corpus callosum. In addition, the injections extended from the rostral portion of the CC anterior mid-body to the caudal portion of the CC posterior mid-body, yet the white matter changes were observed only in the CC posterior mid-body and more posteriorly to

the splenium. Thus, we believe that it is unlikely that the white matter damage could have resulted from a direct impact of the injection needles on the dorsal cortical areas.

Although no changes of the surface area and FA and diffusivity indices were observed in the anterior segments of the CC (genu and rostral body), significant FA reduction and diffusivity increase were observed anteriorly in the white matter fibers of the ventromedial prefrontal. These diffusivity changes in ventromedial prefrontal cortex corroborate the reduction in FA and increase in radial diffusivity (Dr) of the ventromedial prefrontal cortex reported in a prior study of the same animals (Meng et al., 2013). Such white matter changes in the prefrontal cortex could be associated with those reported in the fornix and the uncinate fasciculus (Meng et al., 2013), as both tracts connect the hippocampus to the ventromedial prefrontal cortex (Cavada et al., 2000; Croxson et al., 2005).

Basically 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 eigen values of the diffusion tensor matrix (Da and Dr) indicate different patterns of underlying pathological alterations, such as the disruption and loss of axonal membranes and myelin in the fiber tracts as well as the alterations in the size, density and organization of axons (Le Bihan, 2003; Song et al., 2003; Song et al., 2002). Thus, the surface area reduction in the posterior CC may be associated with a reduction in axonal membranes or myelin as reduced diffuse restriction could result in an increase of diffusivity in the fibers (Le Bihan et al., 2001). In addition, the increased Da could be due to increased extra-axonal space resulting from reduced fiber axonal caliber (Rosas et al., 2010). The microstructural alterations will be validated by post-mortem brain histological analyses that will be performed after all *in vivo* experiments of the Neo-H animals are completed.

Relationships to cognitive deficits

The presence of microstructural alterations in the posterior transcallosal fibers (proximal to posterior parietal and retrosplenial cortices) are also of interest considering the profound memory deficits that were reported previously in the same animals. Both lesion and neuroimaging studies have indicated a significant contribution of the posterior parietal and retrosplenial cortices in navigation and spatial memory processes (Andersen et al., 1985; Harker and Whishaw, 2004; Keene and Bucci, 2009; Vann et al., 2009; Whitlock et al., 2008). Although monkeys with Neo-Hibo showed preserved abilities to solve tasks requiring memory of spatial locations relative to a subject (Heuer and Bachevalier, 2011a; Lavenex et al., 2007), they are severely impaired in tasks requiring memory of objects relative to their spatial relationship with other objects or features in the environment (Blue et al., 2013; Glavis-Bloom et al., 2013). Thus, these spatial memory deficits after Neo-Hibo injury may have resulted not only from the hippocampal damage per se, but rather from disruption of the structural and functional interactions between the impaired hippocampus and the parietal and retrosplenial cortices. Similarly, given the important contribution of the ventromedial prefrontal cortex to object memory (see for review (Eichenbaum, 2017)), it is also interesting to note that the Neo-Hibo animals had severe deficits in object recognition

memory (Zeamer and Bachevalier, 2013; Zeamer et al., 2010). Our data showed that FA in transcallosal fiber tracts across splenium significantly correlated with the scores in VPC-delay task, suggesting the association of transcallosal fiber tracts to occipital cortex with recognition memory (Zeamer and Bachevalier, 2013).

Implications to Human Studies

The abnormality in the middle and posterior corpus callosum and hippocampus has been observed in patients with neuropsychological disorders. For instance, reduced volume in middle and posterior callosal areas has been reported in people with Autism Spectrum Disorder (ASD) (Egaas et al., 1995; Piven et al., 1997). Abnormal fiber microstructure in ASD was seen in posterior midbody and isthmus of corpus callosum in a prior DTI study (Keller et al., 2007). Abnormal metabolite concentrations in hippocampal formation in ASD were observed by proton magnetic resonance spectroscopy as well (Suzuki et al., 2010). The developmental disabilities (such as dyslexia in children and schizophrenia) are also associated with microstructural changes in the posterior corpus callosum (Hasan et al., 2012) and altered functional activity in hippocampal gyrus as measured with functional MRI (Temple et al., 2003).

The present findings also suggest that white matter changes in the posterior corpus callosum and alterations of its interactions within the parietal-retrosplenial-hippocampal network may play a role for the cognitive deficits that have been reported in patients with developmental amnesia (Bachevalier and Vargha-Khadem, 2005). Developmental amnesia has been reported in a series of children with perinatal hypoxic injury resulting in bilateral damage to the hippocampus. These children developed profound deficits in declarative memory mostly sparing semantic memory (Adlam et al., 2009; Gadian et al., 2000; Vargha-Khadem et al., 1997), and, similarly to the memory deficits observed in the Neo-H monkeys, the human cases with early hippocampal insult also showed impaired novelty preference (Munoz et al., 2011), spatial memory (Kessels et al., 2001; King et al., 2000; Piekema et al., 2007) as well as working memory (Geva et al., 2016). Yet, little is known about structural abnormalities outside the hippocampus associated with developmental amnesia in humans. A recent neuroimaging investigation of diencephalic damage (thalamus and mammillary bodies) in 18 patients with developmental amnesia and 18 controls reported a marked degree of atrophy in these two diencephalic structures (Dzieciol et al., 2017). Interestingly, Cormack and colleagues (Cormack et al., 2005) reported extra-hippocampal grey matter density abnormalities in pediatric mesial temporal sclerosis, including reduced grey matter in the thalamus as well as posterior cingulate and parietal opercular cortices that are likely associated with alterations of transcallosal fibers proximal to parietal and retrosplenial cortices similar to those described here for the Neo-H monkeys. These extra-hippocampal changes may be caused by the disruption of normal cortical development as a result of the loss of functional inputs from the damaged hippocampus.

Conclusion

The present DTI study reveals the impact of neonatal hippocampal lesions on the integrity of specific corpus callosum segments in adulthood using a macaque monkey model. The

findings demonstrate that the neonatal hippocampal lesions caused alterations in the posterior corpus callosum and transcallosal fibers proximal to parietal and retrosplenial cortices, and hemispheric connections through posterior corpus callosum. As white matter plays a critical role for information transfer within the brain, the findings may provide complementary information for understanding the neural substrate of behavioral and cognitive deficits observed in patients with early insult to the hippocampus.

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Figure 1.

Sagittal T1-weighted images through the anterior-posterior portion of the hippocampal formation in adult monkeys. Left column illustrates the hippocampus on the right hemisphere of a sham-operated control (Neo-C-1, white arrows). Middle and right columns illustrate the volume reduction of the left and right hippocampus (*) in one adult macaque monkey with neurotoxic hippocampal lesions (Neo-Hibo-2).

Figure 2.

TBSS results reveal significant changes (red color) of DTI indices in different regions of corpus callosum of adult monkeys with Neo-Hibo and the transcallosal fiber tracts delineated by probabilistic tractography (yellow color).

Row a: sagittal T1-weighted images displaying: 1) white vertical lines marking on the most left column representative axial slice locations displayed in Row b-f; 2) areas of altered FA, MD, Da, and Dr (respectively) from next left to the most right columns; and 3) vertical lines on second from right image marking PMb, IS and SP segments in posterior CC.

Row b-f: reduced FA, increased MD, Da, and Dr overlaid on representative axial slices of the FA template. Areas in red indicate significant group differences, including reduced FA and increased diffusivities (MD, Da and Dr), evaluated with standard two-sample independent t-test with FDR correction. To aid visualization, voxels showing significant group differences are thickened using the `tbss_fill` script implemented in FSL. Green color indicated the skeletonized maps of the DTI-derived measures. Coronal images (b-f) are taken from the anterior-posterior levels shown on the sagittal section in (a). Abbreviations. CC: corpus callosum; PMb: posterior midbody; IS: isthmus (IS); SP: splenium; MD: mean diffusivity; Da: axial diffusivity; Dr: radial diffusivity. Neo-Hibo: neonatal hippocampal lesions.

Figure 3. Correlations between mean diffusivity (MD) values and the surface areas (mm²) of the posterior midbody (PMb) in animals with neonatal hippocampal lesions. ($p < 0.05$, Pearson's correlation).

Figure 4. Correlations between FA of transcallosal fiber tracts of splenium with percent correct recognition scores for animals with neonatal hippocampal lesions ($p < 0.05$, Pearson's correlation).

Table 1

Group differences of the surface areas (mm^2 , mean $\pm$ standard derivation) in the segmented corpus callosum of adult sham-operated control monkeys (Neo-C) and received neurotoxic lesions in hippocampus (Neo-Hibo), analyzed with two-sample independent t-test.

	Neo-C	Neo-Hibo	p value
Rostrum	1.51 $\pm$ 0.47	1.66 $\pm$ 0.42	0.61
Genu	20.03 $\pm$ 3.87	17.51 $\pm$ 2.89	0.28
Rostral body	13.57 $\pm$ 2.44	12.00 $\pm$ 2.59	0.35
Anterior midbody	11.66 $\pm$ 1.45	10.57 $\pm$ 2.20	0.38
Posterior midbody	8.85 $\pm$ 1.56	7.06 $\pm$ 0.77	0.05
Isthmus	8.38 $\pm$ 1.36	7.73 $\pm$ 0.60	0.35
Splenium	20.31 $\pm$ 2.11	19.27 $\pm$ 1.61	0.41

Table 2

Pearson's correlations between segmented corpus callosum surface areas and the DTI-derived measures for corpus callosum with significant group difference in animals receiving neurotoxic neonatal lesions in hippocampus.

	Posterior Midbody			Isthmus	Splenum	
	MD	Da	Dr	MD	Da	Da
r	-0.94	-0.67	-0.77	-0.48	-0.35	-0.64
p	0.01 *	0.11	0.07	0.21	0.28	0.12

*
p < 0.05

Table 3

Pearson's correlations (* $p < 0.05$) between memory performance and the DTI indices of corpus callosum/associated transcallosal fiber tracts in animals with neonatal hippocampal lesions.

Areas		Self-order		VPC-delay		VPC-spatial	
		r	p	r	p	r	P
Posterior Midbody	MD	-0.77	0.06	-0.37	0.32	0.44	0.23
	Da	-0.12	0.43	-0.69	0.16	-0.50	0.20
	Dr	-0.41	0.25	-0.64	0.18	0.18	0.39
Isthmus	MD	-0.12	0.42	0.82	0.09	-0.29	0.32
	Da	-0.53	0.18	-0.65	0.18	0.33	0.29
Splenium	Da	-0.37	0.27	-0.69	0.16	0.34	0.29
Tracts across Posterior Midbody	FA	0.46	0.22	0.57	0.22	-0.22	0.36
	MD	-0.70	0.10	0.51	0.24	0.70	0.10
	Da	-0.47	0.21	0.36	0.32	0.32	0.30
	Dr	-0.10	0.44	-0.13	0.44	0.36	0.28
Tracts across Isthmus	FA	0.40	0.25	0.54	0.23	0.25	0.34
	MD	-0.10	0.44	0.31	0.34	0.43	0.23
	Da	-0.46	0.22	-0.25	0.38	0.13	0.42
	Dr	0.54	0.17	0.20	0.40	-0.40	0.25
Tracts across Splenium	FA	-0.30	0.31	0.91	0.04*	0.77	0.07
	MD	-0.19	0.38	-0.90	0.05*	0.12	0.42
	Da	0.37	0.27	-0.22	0.39	-0.68	0.11
	Dr	-0.53	0.18	-0.60	0.20	0.02	0.49