Neuroscience Letters 480 (2010) 93–96

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The role of early life stress in development of the anterior limb of the internal capsule in nonhuman primates

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ARTICLE INFO

Article history:
Received 12 April 2010
Received in revised form 24 May 2010
Accepted 3 June 2010

Keywords:
Diffusion tensor imaging
Fractional anisotropy
White matter integrity
Variable foraging demand

ABSTRACT

Deep brain stimulation (DBS) of the anterior limb of the internal capsule (ALIC) may be effective in treating depression. Parental verbal abuse has been linked to decreased fractional anisotropy (FA) of white matter and reduced FA correlated with depression and anxiety scores. Utilizing a nonhuman primate model of mood and anxiety disorders following disrupted mother–infant attachment, we examined whether adverse rearing conditions lead to white matter impairment of the ALIC. We examined white matter integrity using Diffusion Tensor Imaging (DTI) on a 3T-MRI. Twenty-one adult male Bonnet macaques participated in this study: 12 were reared under adverse [variable foraging demand (VFD)] conditions whereas 9 were reared under normative conditions. We examined ALIC, posterior limb of the internal capsule (PLIC) and occipital white matter. VFD rearing was associated with significant reductions in FA in the ALIC with no changes evident in the PLIC or occipital cortex white matter. Adverse rearing in monkeys persistently impaired frontal white matter tract integrity, a novel substrate for understanding affective susceptibility.

Several lines of evidence link the pathophysiology of mood and anxiety symptoms to white matter abnormalities. For instance, macroscopic white matter lesions that affect prefrontal-subcortical circuits are highly associated with post-stroke depression [26,27]. Diffusion tensor imaging (DTI), a modality of MRI technology, quantifies fractional anisotropy (FA), a measure of the directionality of diffusion of water molecules within white matter tracts of the brain. FA is relatively high in white matter, and is reduced in demyelinating conditions [2]. Reduced FA in the absence of gross pathology of white matter may represent a subtle impairment of normative structural organization of axons [10]. Molecular abnormalities in frontal white matter tracts are observed in late-life depression [20] and adolescent bipolar disorder [1], suggesting an association of white matter changes with depression independent of age and depressive subtype. Moreover, treatment with electroconvulsive therapy in late-life depression normalizes FA of frontal white matter tracts [20].

Deep brain stimulation (DBS) of white matter, particularly the anterior limb of the internal capsule (ALIC) and the closely associated subcallosal cingulate white matter may be effective in treating depression [13]. Using DTI tractography techniques, the ALIC demonstrates widespread projections to frontal pole, medial temporal lobe, cerebellum, nucleus accumbens, thalamus, hypothalamus, and brainstem [13]. Moreover, the ALIC has been

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doi:10.1016/j.neulet.2010.06.012

Table 1
Fractional anisotropy assessments: independent variables and effects of rearing. There was a significant rearing effect on FA values in the anterior limb of the internal capsule, with VFD-reared animals having significantly lower FA than non-VFD animals. The effect of rearing was sufficiently robust that it was still significant even after controlling for the three ROIs examined in this report ($p = .013$ after Bonferroni correction).

	Non-VFD rearing ($N = 8$)[a]	VFD rearing ($N = 12$)	t-Value	df	p
Age (lunar months)	62 ± 31	60 ± 32	0.13	18	0.895
Weight (kg)	5 ± 1	5 ± 1	−0.09	18	0.930
Brain volume (mm^3)	76662 ± 5207	81478 ± 6201	−1.81	18	0.087
White matter volume (mm^3)	3906 ± 476	3978 ± 506	−0.32	18	0.754
Anterior limb of the internal capsule	142 ± 35	101 ± 26	3.02	18	0.007
Posterior limb of the internal capsule	240 ± 39	229 ± 38	0.58	18	0.566
Occipital lobe white matter	276 ± 36	272 ± 60	0.16	18	0.873

VFD = variable foraging demand.
[a] One non-VFD outlier excluded.

reported to be involved in multiple psychiatric disorders, particularly schizophrenia [19,24,25,29,30] and obsessive compulsive disorder [3,9]. Furthermore, the ALIC is involved in two important circuits, the medial and the basolateral limbic circuits [29]. Thus, abnormalities of the ALIC may result in a dysfunction of the connectivity between the temporal and frontal regions as well as in an impairment of neural circuits involved in depression. To our knowledge, there is no report that examines the integrity of white matter of the ALIC in association with early life stress either in humans or in translational animal models.

More recently, using DTI, parental verbal abuse was found to reduce integrity of white matter in the (1) left superior temporal gyrus, (2) cingulum bundle by the posterior tail of the left hippocampus, and (3) the left body of the fornix. White matter integrity in these areas was strongly associated with parental verbal abuse. Fractional anisotropy in region 2 was inversely associated with ratings of depression, whereas fractional anisotropy in region 3 was inversely correlated with ratings of anxiety [4]. These data support the view that early life stressors may impair white matter integrity with affective consequences.

In the nonhuman primate, we have previously modeled persistent susceptibility to anxiety and mood disorders following early life stressors [7]. In bonnet macaques, exposure to early life stress in the infant is achieved through imposing unpredictable foraging conditions on the mother—a procedure termed "variable foraging demand" (VFD). VFD rearing disrupts normative patterns of maternal rearing and infant attachment, and exposed infants exhibit persistent abnormalities in neuroanatomical and neurochemical systems implicated in the etiology of mood and anxiety disorders [7,17,18,21]. Animal models can complement human studies by experimental control of early life stress while also providing a post-stress environment free of confounding variables [12].

Human myelination of the internal capsule progresses rostrally. Most of the myelination in the posterior limb of the internal capsule is complete shortly after birth, whereas myelination of the anterior limb of the internal capsule is ongoing until about a year postnatally (4–5 months on T1W MRI and 8–12 months on T2W MRI)—approximately the period in development when the VFD paradigm was implemented [8]. There is a paucity of data on white matter maturation during the first year of life in nonhuman primates. However, those studies that have examined postnatal brain development in rhesus monkeys showed a similarity in white matter mylelination and growth between human and nonhuman primates with the exception that white matter growth was 3–4 times faster in monkeys [16]. The purpose of the current study was to examine white matter integrity of the anterior limb of the internal capsule using DTI, given its emerging role in human mood and anxiety states [28]. We hypothesized that early life stress may affect white matter formation in nonhuman primates and would be associated with reduced FA in the anterior limb of the internal capsule.

Twenty-one adult male bonnet macaques (*Macaca radiata*) served as subjects: 12 raised under VFD conditions, and nine raised under normative conditions. The subjects were approximately 5 years at time of scanning (Table 1).

Subjects were socially-housed in the SUNY-Downstate Nonhuman Primate Facility. The study was approved by the Institutional Animal Care and Use Committees of SUNY-Downstate, Mount Sinai School of Medicine (MSSM), and Yale University School of Medicine.

VFD procedures are described in detail elsewhere [6]. Briefly, mother–infant dyads were group-housed in pens of 5–7 dyads each and stabilized for at least 4 weeks prior to VFD onset. After infants reached at least 2 months of age, dyads were subjected to a standard VFD procedure that involved eight alternating 2-week blocks in which maternal food was either readily obtained (low foraging demand (LFD) or easy phase) or more difficult to access (high foraging demand (HFD) phase). Difficulty in obtaining food for the mothers was achieved through the use of a foraging cart, a device in which food rations can be hidden in wood chips. No caloric restriction is present in the VFD procedure [22]. Following the VFD procedures, offspring were first housed with their mothers and then in standard peer social groups once they reached the juvenile phase of development, with no subsequent experimental manipulations that could confound the VFD-rearing effects.

As described in detail in a previous report [18], scans were completed at Mount Sinai Medical Center imaging facility, with the monkey's head positioned in a Styrofoam headrest and the anesthetic Saffan used to minimize movement artifact.

DTI data were acquired on a 3 T MRI Siemens Scanner. The protocol for the structural scans consisted of a three-plane sagittal localizer from which all other structural scans were prescribed. The following structural scans were acquired: Axial 3D-MPRage (TR = 2500 ms, TE = 4.4 ms, FOV = 21 cm, matrix size = 256 × 256, 208 slices with thickness = 0.82 mm); Turbo spin-echo T2-weighted Axial (TR = 5380 ms, TE = 99 ms, FOV = 18.3 cm × 21 cm, matrix = 512 × 448, Turbo factor = 11, 28 slices, thickness = 3 mm skip 1 mm); DTI using a pulsed-gradient spin-echo sequence with EPI-acquisition (TR = 4100 ms, TE = 80 ms, FOV = 21 cm, matrix = 128 × 128, 24 slices, thickness = 3 mm skip 1 mm, b-factor = 1250 s/mm^2, 12 gradient directions, averages). Raw DTI data were transferred to an off-line workstation for post-processing. In-house software written in *Matlab* v6.5 (The Mathworks Inc. Natick, MA) was used to compute the anisotropy and vector maps. The NEX is five and eddy-current correction was performed using an adaptation of Camino/SPM package [5]. The Fractional Anisotropy (FA) images were then converted to analyze format. In-house developed software on the Matlab platform was used to access regions of interest (ROI) values of the FA images. As depicted in Fig. 1, ROIs included the anterior and posterior limbs of the internal capsule and included left and right occipital lobe white matter. Care was taken in obtaining the axial DTI scans parallel to the AC-PC line. The anatomical FA images were surveyed that