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The role of early life stress in development of the anterior limb of the internal capsule in non-human primates

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Abstract

Background—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.

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Methods—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.

Results—VFD rearing was associated with significant reductions in FA in the ALIC with no changes evident in the PLIC or occipital cortex white matter.

Conclusion—Adverse rearing in monkeys persistently impaired frontal white matter tract integrity, a novel substrate for understanding affective susceptibility.

Keywords

Diffusion tensor imaging; fractional anisotropy; white matter integrity; variable foraging demand

Introduction

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 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 myelination 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.

Materials and Methods

Subjects

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 five 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

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 four 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.

Diffusion tensor imaging

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 = 4.4ms, 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 = 18.3×21 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/mm², 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 Figure 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 cut across the striatum and a most center slice was selected that showed the anterior and posterior portion of the internal capsule.

Total brain volume

Images were processed using the voxel-based morphometry (VBM) methodology as implemented with the VBM Toolbox into SPM5 (dbm.neuro.uni-jena.de/vbm). The sum of all voxel values within the segmented image approximates total volume within the corresponding partition. Total brain volume was computed from the sum of grey and white matter volume.

Data analysis

Data were first inspected for outliers. Analyses of Variance (Statistica 7.0) were conducted for the three regions of interest. As there were no laterality effects, only FA values for the mean anterior limb of the internal capsule, posterior limb of the internal capsule, and occipital lobe white matter are reported. Age, weight, white matter volume, and whole brain volume were examined as covariates. However, despite the observation of a trend of increased whole brain volume in the VFD vs. the control group, none of the covariates were related to the group outcome measures and were therefore not used. Post hoc t-tests were performed for each region of interest. A Bonferroni correction was used to control for the three areas examined, such that $p < 0.013$ was deemed significant, two tailed.

Results

One non-VFD outlier was noted for the right anterior limb of the internal capsule. The outlier value was 34.5, the non-VFD group mean 133.99 and standard deviation = 21.13. The outlier therefore was 4.7 standard deviations from its group mean and this case was excluded from the analysis. For independent variables, there were no group differences for age, weight, brain volume or white matter volume (Table 1).

For dependent variables, for the anterior limb of the internal capsule, there was a significant rearing effect, with VFD-reared animals having significantly lower FA than non-VFD animals. The results of the ROI analyses are depicted in Table 1. The effect of rearing was significant after controlling for the three ROIs using Bonferroni correction. For the posterior limb of the internal capsule and occipital lobe white matter, as indicated in Table 1, there were no significant effects of rearing.

The data therefore suggest that early life stress in nonhuman primates persistently impairs white matter integrity in the anterior limb of the internal capsule.

Discussion

In general accordance with human studies of parental verbal abuse, monkeys reared under adverse VFD conditions had reduced FA confined to the anterior limb of the internal capsule (ALIC) but not in posterior limb of the internal capsule (PLIC) or occipital white matter. Early life stress may affect axon diameter, microtubular structure, and the proportion of myelinated and unmyelinated fibers that constitute a component of the pathway [15]. The possibility is raised that windows of vulnerability may determine regional reductions in FA [8]. Knowing that most of the PLIC myelination is complete by birth [8,11], we surmised that the PLIC window of vulnerability anteceded the VFD procedures. This may have contributed to the null findings with respect to the PLIC. On the other hand, studies in rhesus monkeys and in humans showed that ALIC maturation continued beyond the age of 2-3 months [8,11,16] – approximately the age of initiating the VFD procedures, which potentially contributed to the FA reduction in the ALIC

Since several psychiatric disorders are associated with white matter defects, a recent study hypothesized that oligodendrocyte abnormalities underlie some aspects of these diseases. The investigators analyzed transgenic mice in which signaling of a myelin-related gene *erbB* is blocked in oligodendrocytes *in vivo*. Changes in oligodendrocyte number and morphology were accompanied by reduced myelin thickness, slower conduction velocity in CNS axons and behavioral alterations [23]. Several caveats warrant mention, the DTI scans were performed only in male subjects, limiting generalizability to females, in whom anxiety and mood disorders are more common [14]. While this homogeneity increases our confidence in the findings from this small sample, future studies with a larger number of subjects should include females. However, to our knowledge, gender differences for ALIC FA have not been reported. Recent DTI studies exploring sites where DBS is applied, have utilized tractography to delineate fiber tracts that traverse the extent of the ALIC seeding [13]. However, because of the limited time of the scan anesthesia in nonhuman primates, during which proton magnetic resonance imaging was also performed, tractography was not feasible. Another limitation of this study was the lack of behavioral data on this cohort.

In summary, the findings suggest that early life stress may persistently impair integrity of white matter tracts of the ALIC. In light of the ALIC being a preferred site for DBS, these findings may suggest the structure bears relevance to the pathophysiology of mood disorders. Neurohistological studies correlating *in-vivo* imaging with *ex-vivo* cytoarchitecture are warranted.

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References

- Adler CM, Adams J, DelBello MP, Holland SK, Schmithorst V, Levine A, Jarvis K, Strakowski SM. Evidence of white matter pathology in bipolar disorder adolescents experiencing their first episode of mania: a diffusion tensor imaging study. *American Journal of Psychiatry*. 2006; 163:322–324. [PubMed: 16449490]
- Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed*. 2002; 15:435–455. [PubMed: 12489094]
- Cannistraro PA, Makris N, Howard JD, Wedig MM, Hodge SM, Wilhelm S, Kennedy DN, Rauch SL. A diffusion tensor imaging study of white matter in obsessive-compulsive disorder. *Depression and Anxiety*. 2007; 24:440–446. [PubMed: 17096398]
- Choi J, Jeong B, Rohan ML, Polcari AM, Teicher MH. Preliminary evidence for white matter tract abnormalities in young adults exposed to parental verbal abuse. *Biological Psychiatry*. 2009; 65:227–234. [PubMed: 18692174]
- Cook P, Bai Y, Nedjati-Gilani S, Seunarine K, Hall M, Parker G, Alexander D. Camino: open-source diffusion-MRI reconstruction and processing. 2006:2759.
- Coplan JD, Altemus M, Mathew SJ, Smith EL, Sharf B, Coplan PM, Kral JG, Gorman JM, Owens MJ, Nemeroff CB, Rosenblum LA. Synchronized maternal-infant elevations of primate CSF CRF concentrations in response to variable foraging demand. *CNS Spectrums*. 2005; 10:530–536. [PubMed: 16155510]
- Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroff CB. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proceedings of the National Academy of Sciences of the U S A*. 1996; 93:1619–1623.
- Cowan FM, de Vries LS. The internal capsule in neonatal imaging. *Seminars in Fetal and Neonatal Medicine*. 2005; 10:461–474. [PubMed: 16002354]
- Duran FL, Hoexter MQ, Valente AA Jr, Miguel EC, Busatto GF. Association between symptom severity and internal capsule volume in obsessive-compulsive disorder. *Neuroscience Letters*. 2009; 452:68–71. [PubMed: 19150484]
- Foong J, Symms MR, Barker GJ, Maier M, Miller DH, Ron MA. Investigating regional white matter in schizophrenia using diffusion tensor imaging. *Neuroreport*. 2002; 13:333–336. [PubMed: 11930133]
- Gibson K. Myelination and behavioral development: A comparative perspective on questions of neoteny, altriciality and intelligence. *Brain maturation and cognitive development: Comparative and cross-cultural perspectives*. 1991:29–63.
- Gorman JM, Mathew S, Coplan J. Neurobiology of early life stress: nonhuman primate models. *Seminars in Clinical Neuropsychiatry*. 2002; 7:96–103. [PubMed: 11953933]
- Gutman DA, Holtzheimer PE, Behrens TE, Johansen-Berg H, Mayberg HS. A tractography analysis of two deep brain stimulation white matter targets for depression. *Biological Psychiatry*. 2009; 65:276–282. [PubMed: 19013554]
- Kendler KS, Kuhn JW, Prescott CA. Childhood sexual abuse, stressful life events and risk for major depression in women. *Psychological Medicine*. 2004; 34:1475–1482. [PubMed: 15724878]
- Keshavan MS, Diwadkar VA, DeBellis M, Dick E, Kotwal R, Rosenberg DR, Sweeney JA, Minshew N, Pettegrew JW. Development of the corpus callosum in childhood, adolescence and early adulthood. *Life Sciences*. 2002; 70:1909–1922. [PubMed: 12005176]
- Malkova L, Heuer E, Saunders RC. Longitudinal magnetic resonance imaging study of rhesus monkey brain development. *Eur J Neurosci*. 2006; 24:3204–3212. [PubMed: 17156381]
- Mathew SJ, Coplan JD, Smith EL, Scharf BA, Owens MJ, Nemeroff CB, Mann JJ, Gorman JM, Rosenblum LA. Cerebrospinal fluid concentrations of biogenic amines and corticotropin-releasing factor in adolescent non-human primates as a function of the timing of adverse early rearing. *Stress*. 2002; 5:185–193. [PubMed: 12186681]

18. Mathew SJ, Shungu DC, Mao X, Smith EL, Perera GM, Kegeles LS, Perera T, Lisanby SH, Rosenblum LA, Gorman JM, Coplan JD. A magnetic resonance spectroscopic imaging study of adult nonhuman primates exposed to early-life stressors. *Biological Psychiatry*. 2003; 54:727–735. [PubMed: 14512213]
19. Mitelman SA, Torosjan Y, Newmark RE, Schneiderman JS, Chu KW, Brickman AM, Haznedar MM, Hazlett EA, Tang CY, Shihabuddin L, Buchsbaum MS. Internal capsule, corpus callosum and long associative fibers in good and poor outcome schizophrenia: a diffusion tensor imaging survey. *Schizophrenia Research*. 2007; 92:211–224. [PubMed: 17329081]
20. Nobuhara K, Okugawa G, Minami T, Takase K, Yoshida T, Yagyu T, Tajika A, Sugimoto T, Tamagaki C, Ikeda K, Sawada S, Kinoshita T. Effects of electroconvulsive therapy on frontal white matter in late-life depression: a diffusion tensor imaging study. *Neuropsychobiology*. 2004; 50:48–53. [PubMed: 15179020]
21. Rosenblum LA, Coplan JD, Friedman S, Bassoff T, Gorman JM, Andrews MW. Adverse early experiences affect noradrenergic and serotonergic functioning in adult primates. *Biological Psychiatry*. 1994; 35:221–227. [PubMed: 8186327]
22. Rosenblum LA, Pauly GS. The effects of varying environmental demands on maternal and infant behavior. *Child Development*. 1984; 55:305–314. [PubMed: 6705632]
23. Roy K, Murtie JC, El-Khodori BF, Edgar N, Sardi SP, Hooks BM, Benoit-Marand M, Chen C, Moore H, O'Donnell P, Brunner D, Corfas G. Loss of erbB signaling in oligodendrocytes alters myelin and dopaminergic function, a potential mechanism for neuropsychiatric disorders. *Proceedings of the National Academy of Sciences of the U S A*. 2007; 104:8131–8136.
24. Sussmann JE, Lymer GK, McKirdy J, Moorhead TW, Maniega SM, Job D, Hall J, Bastin ME, Johnstone EC, Lawrie SM, McIntosh AM. White matter abnormalities in bipolar disorder and schizophrenia detected using diffusion tensor magnetic resonance imaging. *Bipolar Disorder*. 2009; 11:11–18.
25. Suzuki M, Zhou SY, Hagino H, Takahashi T, Kawasaki Y, Nohara S, Yamashita I, Matsui M, Seto H, Kurachi M. Volume reduction of the right anterior limb of the internal capsule in patients with schizotypal disorder. *Psychiatry Research*. 2004; 130:213–225. [PubMed: 15135156]
26. Vataja R, Leppavuori A, Pohjasvaara T, Mantyla R, Aronen HJ, Salonen O, Kaste M, Erkinjuntti T. Poststroke depression and lesion location revisited. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 2004; 16:156–162. [PubMed: 15260366]
27. Vataja R, Pohjasvaara T, Leppavuori A, Mantyla R, Aronen HJ, Salonen O, Kaste M, Erkinjuntti T. Magnetic resonance imaging correlates of depression after ischemic stroke. *Archives of General Psychiatry*. 2001; 58:925–931. [PubMed: 11576030]
28. Wobrock T, Kasper T, Roy A, Vogetley K, Schneider-Axmann T, Wagner M, Maier W, Rietschel M, Schulze TG, Scherk H, Schild HH, Block W, Traber F, Tepest R, Honer WG, Falkai P. Reduction of the internal capsule in families affected with schizophrenia. *Biological Psychiatry*. 2008; 63:65–71. [PubMed: 17574215]
29. Zhou SY, Suzuki M, Hagino H, Takahashi T, Kawasaki Y, Nohara S, Yamashita I, Seto H, Kurachi M. Decreased volume and increased asymmetry of the anterior limb of the internal capsule in patients with schizophrenia. *Biological Psychiatry*. 2003; 54:427–436. [PubMed: 12915287]
30. Zou LQ, Xie JX, Yuan HS, Pei XL, Dong WT, Liu PC. Diffusion tensor imaging study of the anterior limb of internal capsules in neuroleptic-naïve schizophrenia. *Academic Radiology*. 2008; 15:285–289. [PubMed: 18280926]

Figure 1. DIFFUSION TENSOR IMAGING OF THE NONHUMAN PRIMATE: ROI VOXEL PLACEMENT

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. 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 Fractional Anisotropy images were then converted to analyze format. MEDx v3.4.3 software (Medical Numerics Inc, Sterling, VA) was used to inspect and define ROIs on the FA images. Occipital lobe was examined as a control region, with no differences expected in this area.

Fractional Anisotropy Assessments: Independent Variables and Effects of Rearing

Table 1

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)*	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

* one non-VFD outlier excluded. VFD = Variable Foraging Demand