Research report

Experience-dependent asymmetric variation in primate prefrontal morphology

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Abstract

Theories of human development suggest that experiences embedded in social relationships alter prefrontal brain systems that mediate emotional self-regulation. This study tests for experience-dependent effects on prefrontal gray and white matter volumes determined in 39 young adult monkeys (Saimiri sciureus) 4 years after conditions that modified early maternal availability. These conditions were previously shown to alter subsequent measures of emotional behavior, social propensities, and hypothalamic–pituitary–adrenal axis stress physiology. Here we identify significant differences in right but not left adult prefrontal volumes, with experience-dependent asymmetric variation most clearly expressed in ventral medial cortex measured in vivo by magnetic resonance imaging (MRI). Follow-up studies now need to determine whether maternal availability directly affects or interacts with subsequent experiences to alter prefrontal substrates of emotional processing and sensitivity to stress. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Growth and development of the prefrontal cortex extends across childhood into early adulthood in human and nonhuman primates [5,6,24,43,77]. This process corresponds with delayed maturation of attention, planning, cognitive control, and emotional self-regulation [17,25,44,52,62,78]. Based on indications that early experiences alter brain systems in rodents [26,42,55,70], theories of human development suggest that stressful experiences in social relationships modify prefrontal maturation [75].

Aside from a limited number of reports on inherited variation in autonomic activity [80] and cerebrospinal fluid (CSF) monoamine concentrations [12,32], primate research on stress neurobiology has focused on severe forms of maternal deprivation. Rhesus macaque monkeys raised without mothers exhibit increased brain dopamine and norepinephrine sensitivity [40,45], exaggerated hypothalamic–pituitary–adrenal (HPA) responses to stress [9,31], altered regulation of autonomic activity [53], fragmented sleep patterns [37], depression-like behavior [40], and excessive consumption of alcohol [20]. Ecologically informed research on maternal availability has likewise identified untoward effects on primate postnatal development. Bonnet macaque monkeys raised by mothers in variable foraging conditions are impaired in social and emotional development [2,69]. These same monkeys exhibit in early adulthood elevated CSF concentrations of monoamines, somatostatin, and corticotropin-releasing factor (CRF) [14,15]. Similar changes in stress neurobiology have been implicated in human psychiatric disorders that are triggered or aggravated by stress [11,54,73].
Childhood stress increases the risk of developing adult mood and anxiety disorders [28], and smaller than normal prefrontal volumes are evident in psychotic depression [39]. Neuroimaging-based volumes of left gray matter tissue ventral to the genu of the corpus callosum are diminished in familial forms of depression and bipolar disorder [19]. Histological investigations of this prefrontal subregion indicate that humans with mood disorders have significantly fewer glia relative to healthy humans, without corresponding reductions in neuron number or size [57]. Reductions in cortical thickness, diminished neuron size, and fewer neurons and glia have all been reported in other prefrontal subregions in humans diagnosed with major depression [65].

Opportunities for research on the causes and consequences of differences in prefrontal morphology are limited in clinical studies of humans. Therefore, we examined early life stress and inherited variation in prefrontal gray and white matter volumes in monkeys. Paternal half-siblings raised apart from one another by different mothers in the absence of fathers were initially randomized to conditions that modified aspects of maternal availability. In one condition on five occasions offspring were briefly separated from groups comprised of three or four mother–infant pairs. Brief intermittent separations provoke repetitive peep-calls, locomotor agitation, and acute elevations in the stress hormone cortisol with baseline levels of these measures restored soon after each social reunion [13,29,79]. In two other postnatal rearing conditions differences in maternal availability were produced by manipulating the effort required to find food. Body weights and amounts of food consumed in each foraging condition were not significantly different, but mothers maintained in the high-demand condition spent 60% more time foraging for food. High-demand mothers stopped carrying infants earlier, and these infants displayed modest prolonged increases in cortisol throughout the high-demand condition [48].

Following completion of each 12 week condition at 21 weeks of age, all mothers were removed from natal groups after weaning at 36 weeks. At this stage of development squirrel monkeys are no longer reliant on maternal care. Sexual maturity is achieved at 3 years, and the squirrel monkey life span is approximately 21 years [7]. As indicated elsewhere in greater detail [49], monkeys exposed to intermittent separations respond to the removal of all mothers after weaning with smaller increases in cortisol levels, fewer distress peep-calls, and more time spent near peers [49]. Monkeys from the low- and high-demand conditions do not differ on any of these measures. In early adulthood at 5 years of age, only the intermittently separated monkeys show signs of enhanced glucocorticoid negative-feedback regulation of the HPA-axis response to stimulation by exogenous CRF [50].

Recently, we found that rearing conditions do not affect subsequent adult hippocampal volumes determined by magnetic resonance imaging (MRI) [51]. The hippocampus is known to be critically involved in regulating the HPA-axis stress response, but other brain regions also play a role, including the prefrontal cortex [18,30,36]. Here we test for rearing-related differences in three prefrontal subregions.

2. Method

2.1. Subjects

Forty infants sired by fathers that had no form of contact with their offspring were distributed randomly in groups each comprised of three or four squirrel monkey mother–infant pairs. All monkeys were of Guyanese origin (Saimiri sciureus), and were born and maintained at the Stanford University Primate Facility. One monkey from the low demand condition was excluded for reasons unrelated to the study. Twelve fathers and 30 mothers produced the 39 monkeys that comprised the study cohort. Twenty-one infants (seven males, six females) and 13 mothers were maintained from 10 to 21 weeks postpartum in high demand conditions where food was easy to find. Four natal groups were randomly assigned to each of the following three rearing conditions when infants were 10 weeks of age (range 8–13 weeks).

1) Low foraging demands. A total of 13 infants (seven males, six females) and their 13 mothers were maintained from 10 to 21 weeks postpartum in low demand conditions where food was easy to find. Each group received 600% by weight of their normal daily food intake buried in foraging boards [48]. All 80 holes in the foraging boards contained abundant amounts of food.

2) High foraging demands. A total of 13 infants (seven males, six females) and 13 mothers were maintained from 10 to 21 weeks postpartum in high demand conditions where each group was provisioned with 120% of their daily food intake buried in foraging boards. Many holes in the foraging boards con-
tained little or no food, so more time and effort was required to find food [48].

3) Intermittent Social Separations. A total of 13 infants (six males, seven females) and 13 mothers fed from standard food-hoppers were separated intermittently for five sessions each lasting 5 h in duration. Every other week from 13 to 21 weeks postpartum, each infant was removed one at a time, placed in a cage adjacent to unfamiliar monkeys, and temporarily deprived of all forms of contact with members of the natal group. Upon completion of these protocols at 21 weeks of age, all monkeys were maintained under standard conditions. As previously described elsewhere in greater detail [49], social behavior, emotional responses, and increases in plasma cortisol levels elicited by removing all mothers after weaning were examined at 36 weeks. Shortly thereafter, offspring were housed with two or three animals of the same gender from different postnatal conditions. Approximately 4 years later, in early adulthood (range 3.6–5.9 years of age), a neuroendocrine challenge was administered to test cortisol negative-feedback regulation of the HPA-axis response to exogenous stimulation by CRF [50]. Then 5 weeks later brain images were acquired by high-resolution MRI.

2.3. Brain image acquisition and analysis

MRI was performed on a General Electric Signa 1.5 T scanner. Monkeys were scanned under anesthesia induced by a subcutaneous injection of 20 mg/kg ketamine, 4 mg/kg xylazine, and 0.04 mg/kg atropine. Body temperatures were maintained within the normal range by using a cushioned heat-pad. Ear plugs provided protection from noises generated by the scanner.

The first scan for each monkey was acquired in the sagittal plane with a 2D sequential SPGR pulse sequence: TR = 18 ms, TE = 4 ms, flip angle = 30, NEX = 1, matrix = 256 × 128, FOV = 8 cm, voxel size = 0.5 × 1.0 × 4.0 mm, slice thickness = 4 mm. This initial localizer scan was used to standardize head tilt and rotation by assuring that two external markers (vitamin E capsules in the meatus of each ear) were aligned in the coronal and axial planes. The head was repositioned as required, and another sagittal localizer scan was performed. Head pitch was then standardized against the midsagittal image, with the final scan acquired in the coronal plane. The final scan used for prefrontal measures was a 3D Inversion Recovery-prepared FSPGR pulse sequence: TR = 12 ms, TE = 3 ms, TI = 300 ms, flip angle = 15, NEX = 4, matrix = 256 × 224, FOV = 8 cm, voxel size = 0.31 × 0.36 × 1.00 mm, slice thickness = 1 mm.

Image processing was performed offline with ANALYZE software (Biomedical Imaging Resource, Mayo Foundation) as previously described for human brain imaging research [76]. To minimize inter-scan variation, a Histogram Match function in ANALYZE was used to normalize gray-scale pixel values for each brain against a single standard. A trained human rater blind to the identity of each monkey then measured all prefrontal volumes of interest. Stereological methods were used with ANALYZE software to generate unbiased estimates of prefrontal volumes on the left and right brain side. Sampling parameters were set to yield at least 150 ‘hits’ per measurement, a number previously shown to generate reliable brain volume determinations [27]. For sampling purposes, a grid was superimposed on each brain image with grid placement randomly determined by ANALYZE. All grid points falling directly on brain tissue were identified by the trained human rater. From these determinations ANALYZE generated an unbiased estimate of prefrontal volumes based on the Cavalieri Principle.

The following rules were used to identify rostral, caudal, and inplane boundaries for the prefrontal regions of interest. This in vivo approach was adapted from MRI studies of human prefrontal morphology [67], but falls short of the standard typically achieved by relying on postmortem prefrontal cytoarchitecture. All gray and white matter anterior to the genu of the corpus callosum was considered to be prefrontal tissue. Prefrontal tissue on each brain side was further subdivided into three non-overlapping regions measured for each monkey on seven to nine contiguous coronal slices extending from the genu of the corpus callosum to the frontal pole (Fig. 1). Prefrontal white matter volumes were defined as all white matter tissue on either side of the interhemispheric fissure. Ventral medial prefrontal cortex on each brain side was defined as gray matter located medial to the orbital sulcus, and inferior to a line drawn perpendicular from the interhemispheric fissure to the orbital sulcus. Dorsolateral prefrontal cortex on

Fig. 1. Representative images of prefrontal subregions at 1 mm intervals in adult squirrel monkey brain.
each brain side was defined as gray matter located lateral to the orbital sulcus, and superior to the line drawn perpendicular from the interhemispheric fissure to the orbital sulcus. Dorsolateral and ventral medial regions are complementary parts of the squirrel monkey prefrontal cortex.

To adjust for variation in overall brain size, brain volumes were defined and subsequently measured as all gray and white matter tissue in both hemispheres, including the midbrain superior to the pons. The superior border of the pons was chosen as the point of demarcation because it is easily recognized on MR images of brain [76]. Total brain volume for each monkey was generated from 15 to 16 coronal slices separated by 3 mm gaps. Based on measurements from two trained raters independently scoring the same 13 monkey brains, inter-rater reliabilities expressed as intraclass correlation coefficients ranged from 0.92 to 0.97 (dorsolateral gray matter = 0.95; ventral medial gray matter = 0.92; prefrontal white matter = 0.93; overall brain size = 0.97).

2.4. Data analysis

Early maternal availability (three rearing conditions), paternity (offspring grouped by father), gender, and brain side differences were examined for each of the three prefrontal subregions. Analyses were performed using least squares linear models in the MGLH module of Systat (Evanston, IL). Gender, paternity, and rearing condition were considered between-subjects factors, and brain side was the repeated-measures factor in three separate four factor ANOVAs. The paternity-by-rearing condition, gender-by-rearing condition, and paternity-by-gender-by-rearing condition interactions were not included because most of the fathers failed to produce sufficient numbers of male and female offspring for each postnatal condition in the unbalanced factorial design [49]. Bonferroni corrections were applied to control for multiple comparisons with each test statistic assessed at \( P < 0.0167 \) for the three prefrontal subregions.

Quantitative estimates of heritability were generated from one-way ANOVAs used to assess paternal half-sibling effects [21]. From separate ANOVAs for each region of interest, the between-father mean square minus the within-father mean square was divided by 3.25 (average number of offspring per father), multiplied by 4 (paternal half-siblings share, on average, 25% of their genome by common descent), and divided by the total variance. Under the null hypothesis of no hereditary effect, within- and between-father components of variance are equivalent, and the resulting \( F \)-ratios approximate 1. As the between-father component of variance increases relative to within-father variance, \( F \)-ratios grow larger in the half-sibling analysis and the null hypothesis is rejected. All test statistics were evaluated with two-tailed probabilities, and descriptive statistics are presented as mean ± S.E.M.

3. Results

Males were significantly larger than females as determined by multivariate ANOVA (Hotelling’s \( T = 1.87, F(5,33) = 12.33, P < 0.001 \)), but the magnitude of sexual dimorphism differed across measures of brain and body size (Fig. 2). Body weights were 30% greater in males, whereas smaller prefrontal differences were discerned. The unilateral measures for each prefrontal subregion were correlated with differences in total brain volume (\( r = 0.40–0.57 \), df 37, \( P < 0.013 \)), and, therefore, prefrontal measures were divided by brain volume to adjust for brain size variation.

Ventral medial prefrontal cortical volumes revealed a rearing condition-by-brain side interaction for adjusted (\( F(2,24) = 5.56, P = 0.010 \)) and unadjusted (\( F(2,24) = 5.64, P = 0.010 \)) measures (Fig. 3). Subsequent analysis of simple main effects confirmed that rearing-related differences were evident in the right, but not left ventral medial volumes for adjusted (Table 1) and unadjusted measures (right ventral medial cortex \( F(2,24) = 5.77, P = 0.009 \); left ventral medial cortex \( F(2,24) = 0.21, P = 0.815 \)). Adjusted right volumes in the intermittently separated monkeys were 14% larger than the low-demand monkeys (\( F(1,24) = 10.92, P = 0.003 \)), and 8% larger than the high-demand monkeys (\( F(1,24) = 5.87, P = 0.023 \)). Similar differences were likewise discerned in unadjusted right ventral medial measures (Fig. 3). Monkey from the low- and high-demand conditions did not differ significantly on adjusted or unadjusted measures of ventral medial cortex.

![Fig. 2. Gender differences in brain and body size. Body weights, total brain volumes, and the volumes of three prefrontal subregions are depicted for the left and right brain sides combined (n = 20 males and 19 females, mean ± S.E.M., * \( P < 0.05 \), ** \( P < 0.0167 \)).](image-url)
Rearing condition-by-brain side interactions were also discerned in dorsolateral cortex (adjusted measures $F(2,24) = 5.48$, $P = 0.011$, unadjusted measures $F(2,24) = 6.01$, $P = 0.008$; Fig. 4) and prefrontal white matter volumes (adjusted measures $F(2,24) = 6.47$, $P = 0.006$, unadjusted measures $F(2,24) = 7.01$, $P = 0.004$; Fig. 5). But the rearing condition main effects for adjusted (Table 1) and unadjusted measures from each subregion failed to reach Bonferroni-corrected levels of significance. Right dorsolateral cortical volumes (Fig. 4) and right prefrontal white matter volumes (Fig. 5) were significantly larger in the intermittently separated monkeys relative to monkeys from the low-demand condition, but not the high-demand monkeys.

In addition to finding rearing condition effects, paternity-related differences were evident in adjusted (Table 1) and unadjusted right ventral medial volumes ($F(11,24) = 3.01$, $P = 0.012$). Certain fathers produced monkeys with large right ventral medial volumes, other fathers produced monkeys with smaller right ventral medial volumes, and similar right ventral medial volumes were found among monkeys that shared a common father (Fig. 6). The estimated proportion of genetic variance, i.e. heritability, was 47% for both adjusted and unadjusted right ventral medial volumes. Distributions of adjusted and unadjusted measures for right ventral medial prefrontal volumes were Gaussian in the sample of 39 monkeys, indicating contributions from multiple additive effects on right ventral medial prefrontal size. Heritability estimates for all other prefrontal subregions did not differ significantly from

Table 1
Rearing condition, paternity, and gender main effects for left and right prefrontal measures adjusted for total brain volume variation in 39 adult monkeys

<table>
<thead>
<tr>
<th></th>
<th>Left brain</th>
<th>Right brain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ventral medial gray matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rearing condition</td>
<td>$F(2,24) = 0.15$, $P = 0.861$</td>
<td>$F(2,24) = 5.94$, $P = 0.008$</td>
</tr>
<tr>
<td>Paternity</td>
<td>$F(11,24) = 1.27$, $P = 0.301$</td>
<td>$F(11,24) = 2.87$, $P = 0.015$</td>
</tr>
<tr>
<td>Gender</td>
<td>$F(1,24) = 0.01$, $P = 0.929$</td>
<td>$F(1,24) = 0.09$, $P = 0.764$</td>
</tr>
<tr>
<td><strong>Dorsolateral gray matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rearing condition</td>
<td>$F(2,24) = 0.19$, $P = 0.824$</td>
<td>$F(2,24) = 3.20$, $P = 0.058$</td>
</tr>
<tr>
<td>Paternity</td>
<td>$F(11,24) = 0.71$, $P = 0.720$</td>
<td>$F(11,24) = 1.38$, $P = 0.243$</td>
</tr>
<tr>
<td>Gender</td>
<td>$F(1,24) = 0.08$, $P = 0.777$</td>
<td>$F(1,24) = 3.83$, $P = 0.062$</td>
</tr>
<tr>
<td><strong>Prefrontal white matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rearing condition</td>
<td>$F(2,24) = 1.61$, $P = 0.220$</td>
<td>$F(2,24) = 3.55$, $P = 0.044$</td>
</tr>
<tr>
<td>Paternity</td>
<td>$F(11,24) = 0.33$, $P = 0.970$</td>
<td>$F(11,24) = 1.48$, $P = 0.203$</td>
</tr>
<tr>
<td>Gender</td>
<td>$F(1,24) = 1.41$, $P = 0.247$</td>
<td>$F(1,24) = 4.25$, $P = 0.050$</td>
</tr>
</tbody>
</table>
4. Discussion

The right but not left ventral medial subregion of prefrontal cortex was 8–14% larger in squirrel monkeys exposed to intermittent separations relative to monkeys raised by mothers in either foraging demand condition. This effect size is comparable to measures reported in now classic studies of environmental enrichment and cortical thickness in rats [70]. Experience-dependent asymmetric variation was also discerned in dorsolateral cortex and prefrontal white matter volumes. The rearing condition-by-brain side interaction was significant for each of these prefrontal subregions, but simple main effects for rearing condition failed to reach Bonferroni-corrected levels of significance. Males were significantly larger than females, and differences in total brain volumes were correlated with each of the prefrontal measures. Nevertheless, rearing-related differences were consistently evident in right ventral medial prefrontal volumes whether or not these measures were adjusted for brain volume variation.

The ventral medial subregion measured in monkeys more-or-less corresponds with agranular cortex lacking a well-developed granular layer IV, as indicated in cytoarchitectural studies of the squirrel monkey frontal lobe [68,72]. Spatial aspects and surface features of cortex only roughly coincide with cytoarchitectonic borders [74], and, therefore, comparisons with conventional cortical maps inevitably are imprecise. But anatomical connections and physiological evidence suggest that the region examined in monkeys contains areas homologous to human and rodent prefrontal ventral medial cortex [58,63].

Right frontal regions in humans have been implicated in processing negative emotional experiences and sensitivity to stress. Pharmacological inactivations by intracarotid injections of sodium amobarbital indicate that negative emotions are lateralized toward the right brain side [1]. Lateralized presentations of negative emotional items [81], functional neuroimaging [8], and electroencephalogram recordings [23,38] have linked right anterior brain regions in humans to cortisol secretion, negative emotion, and aspects of social withdrawal. It is, therefore, intriguing that intermittent separations reliably elicit a robust stress response, and subsequently lead, later in life, to increased right prefrontal volumes in monkeys, diminished expression of negative affect, increased affiliative social tendencies toward peers, and enhanced glucocorticoid negative-feedback regulation of the HPA-axis stress response [49,50].

Our findings in monkeys parallel studies of rats indicating that brief intermittent separations diminish emotionality and HPA-axis stress responses throughout adolescence and adulthood [16,42,55]. Diminished stress responses are mediated in part by enhanced glucocorticoid negative-feedback resulting from increased glucocorticoid receptor (GR) expression in the rat hippocampal formation [55,56]. Enduring elevations in hippocampal GR concentrations arise from changes in maternal behavior when rat pups are returned to the nest. High levels of maternal licking permanently increase GR concentrations and enhance sensitivity to glucocorticoid feedback in rats subsequently studied as adults [3,47].

Enhanced glucocorticoid negative-feedback in monkeys is not likely due to maternal licking per se, since squirrel monkey mothers seldom engage infants in bouts of licking or grooming [33]. Monkeys also differ in brain GR distributions relative to the pattern found in rats. Rat brain GR concentrations are greatest in the adult rodent hippocampus, whereas dense GR concentrations are found in primate prefrontal cortex [59,71]. If large right ventral medial prefrontal volumes confer proportionally more GR in the intermittently separated monkeys, then experience-dependent prefrontal maturation may contribute to enhanced regulation of the HPA-axis response. This hypothesis suggests that intermittent social stress triggers adaptations in the postnatal brain [34,47,55], whereas chronic increases in cortisol levels elicited by high-demand foraging in monkeys do not produce these effects.

An obvious limitation of this study is related to reliance on cross-sectional measures collected from young adult monkeys 4 years after manipulations that modify maternal availability at 10–21 weeks of age. We do not yet know whether maternal availability directly affects or interacts with subsequent experiences that lead to right prefrontal enlargement. Longitudinal studies now need to determine when right prefrontal enlargement first emerges, and whether this process is related to aspects of interactive stimulation during social reunions.
The mechanisms that mediate prefrontal enlargement must likewise be considered with caution. Right prefrontal enlargement could reflect systematic differences in neurogenesis, or selective elimination of neurons, dendrites, and synaptic connections. The latter possibility is perhaps more likely since prefrontal volumes normally exhibit a 10% decrease around puberty in humans [24], whereas neuronal and synaptic proliferation is complete soon after parturition in primates [64,66]. Neurogenesis parallels angiogenesis in the visual cortex [22]. In addition to providing metabolic support, recent evidence suggests that endothelial microvasculature supports the survival and maturation of neurons by increasing local levels of non-sequestered brain derived neurotrophic factor (BDNF) [41]. This finding is of interest in light of reports that high levels of maternal licking and arched-back nursing increase the expression of BDNF in rodent postnatal hippocampus [46]. Normative patterns in BDNF gene expression are evident in primate prefrontal cortex [35], but nothing is known about social regulation of BDNF and selective survival of neurons in prefrontal cortex.

A final aspect of this study that warrants comment concerns the similarities in right prefrontal ventral medial volumes found among monkeys that shared a common father. Since paternal half-siblings were raised apart from one another by different mothers in the absence of fathers, phenotypic similarities among paternal half-siblings cannot be attributed to shared family environments. Based on a standard half-sibling analysis, the estimated heritability was 47% for right ventral medial prefrontal size. Comparable heritabilities for overall brain size have been reported in humans [4,60,61] and rhesus macaque monkeys [10], but little is known about the genetics of variation in regional brain morphologies.

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