

Cerebrospinal Fluid Concentrations of Somatostatin and Biogenic Amines in Grown Primates Reared by Mothers Exposed to Manipulated Foraging Conditions

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Background: In an earlier study, infant primates were nursed by mothers randomly assigned to variable foraging demand (VFD) or nonvariable foraging conditions (non-VFD). A group of grown VFD-reared subjects demonstrated elevations of cisternal cerebrospinal fluid (CSF) corticotropin-releasing factor concentrations and decreased CSF cortisol levels vs non-VFD counterparts. To further characterize neurobiological sequelae of disturbed early rearing, CSF concentrations of serotonin, dopamine, and norepinephrine metabolites (5-hydroxyindoleacetic acid, homovanillic acid, and 3-methoxy-4-hydroxyphenethyleneglycol [MHPG], respectively) and of somatostatin were determined.

Methods: Second CSF taps were obtained from the previously studied cohort of 30 subjects and from 28 age-matched ad libitum-reared control subjects. Relevant assays were performed.

Results: All neurochemicals assayed except MHPG were elevated in the VFD-reared compared with non-VFD sub-

jects. In the VFD group, statistically significant positive correlations between corticotropin-releasing factor and each neurochemical was found, except for MHPG. In the non-VFD subjects, no significant correlations with corticotropin-releasing factor were observed. No effect of age was evident.

Conclusions: Reducing the predictability of maternal foraging demand during early rearing was associated with elevations of cisternal somatostatin and of serotonin and dopamine metabolite concentrations in grown offspring. The corticotropin-releasing factor elevations reported previously were positively correlated with all the elevated CSF parameters of the current study. The findings support the notion that adverse early rearing experiences in primates have longstanding and complex effects on a range of neurochemicals relevant to emotional regulation. Replication in prospective age-controlled studies is warranted.

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THE PIVOTAL role of adverse early life experiences in the pathogenesis of adult psychopathological symptoms has long been recognized in both psychoanalytic circles¹ and systematic epidemiologic studies.^{2,3} Primate infants, human and nonhuman, display a relatively extended period of dependence on their mothers during development. Relatively subtle disruptions during critical “windows” of maternal-infant affective interaction may come to symbolize a threat to survival, with long-term behavioral and biological (mal)adaptations.⁴

To study the potential effects of such early disruptions, 3 different foraging paradigms were implemented so that accessibility to food could be mechanically manipulated while the primate mother was nursing her infant.⁵ In the first paradigm,

mothers were exposed to consistently low foraging demand conditions (LFD); ie, food was readily obtained with little time or effort required. The second paradigm involved consistently high foraging demand conditions (HFD). For the third paradigm, referred to as variable foraging demand (VFD), foraging was alternated between low and high demand every 2 weeks. Several reports⁴⁻⁷ have indicated that VFD-reared primate infants show stable behavioral patterns reminiscent of anxiety in human children.

Cisternal taps on adolescent primate subjects indicated that cerebrospinal fluid (CSF) concentrations of the stress-related neuropeptide corticotropin-releasing factor (CRF) were elevated in VFD subjects in comparison with both HFD and LFD groups. The latter 2 groups were indistinguishable from each other.⁸ Despite high levels of CRF in CSF, VFD-

MATERIALS AND METHODS

Thirty bonnet macaques (*Macaca radiata*) served as subjects for the study. For 12 to 14 weeks beginning when the infants were approximately 17 weeks of age, 15 of the subjects (10 male and 5 female) were raised under VFD conditions, 8 (5 male and 3 female) under HFD conditions, and 7 (4 male and 3 female) under LFD conditions. The mean age at the time of the cisternal CSF sampling in the VFD subjects was 4 years, with all HFD and LFD subjects born within a few weeks of each other. As the VFD subjects were significantly younger than the subjects of the other 2 groups, the role of age in CSF studies was explored in a separate control group of 28 monkeys. These subjects were reared by their mothers in laboratory breeding groups under stable ad libitum conditions, generally comparable with LFD. These additional animals had a mean age of 30 months (minimum, 15 months; maximum, 52 months; SD, 10.3 months), spanning the age range of the differentially reared groups. The age range of the animals at the time of CSF sampling corresponded to that of peripubertal to young adult phases of human development.

Rearing treatment was preceded by habituation to the rearing paradigm, in which infants were required to remain within a nursery enclosed by contact-permissive mesh within their mothers' pens. A full range of mother-infant behavioral patterns was permitted by the design. Following habituation to the nursery paradigm, differential treatment began. For the HFD mothers, this consisted of 12 weeks in which they were required to dig through clean wood-chip bedding to obtain their daily food ration. For the LFD mothers, abundant food items could simply be picked up from the pen floor during this same 12-week period. For the VFD mothers, foraging demand varied

between low and high in 2-week blocks during the 12-week rearing period, beginning with 2 weeks of low demand. The low-demand blocks were identical to the LFD condition. During the high-demand blocks, 4 mothers were required to dig for their food as in the HFD condition, whereas 11 mothers were required to perform a joystick-video task described previously⁷ to earn 190-mg banana-flavored food pellets. Mothers were trained on the joystick task prior to nursing. Four infants of this latter group of 11 mothers were not housed in the nursery enclosure. Water was constantly available to all mothers. All infants had free access to food and water in an area inaccessible to the mothers. Weekly body weight measurements and health checks revealed normal growth and health in both mothers and infants of all groups, consistent with the developmental norms of the bonnet macaque determined in this laboratory during a 30-year period. Mothers did not receive differential amounts of food throughout the experimental period. Infants were weaned at age 6 months or older and were completely separated from their mothers at 1 year of age, subsequently living in stable combined (VFD and non-VFD) peer groups until the current studies.^{6,7}

Approval was obtained for all studies from the Institutional Animal Care and Use Committee of both SUNY-Health Sciences Center at Brooklyn and Columbia University, New York, NY. The CSF sampling commenced with the administration of ketamine (10 mg/kg) within 2 minutes after the subject's entrance into a squeeze cage. The suboccipital area was prepared with 2% povidone iodine solution. The animal was placed in a strictly symmetrical sitting position and the neck was fully flexed to expose a small triangular depression directly below the occiput and superficial to the cisterna magna. A 24-gauge, 3/4-in needle with a 3-mL syringe was advanced perpendicular to the surface. Once the dura had been penetrated, 1.5 mL of CSF

reared subjects exhibited lower CSF cortisol levels. High levels of CRF in CSF⁹ in the context of a suppressed hypothalamic-pituitary-adrenal axis¹⁰ strikingly resembles the biochemical profile reported in human post-traumatic stress disorder.

In the identical subjects, we assayed the peptide somatostatin (SOM), whose release is stimulated by CRF¹¹ and which exerts a putative inhibitory influence on the hypothalamic-pituitary-adrenal axis.¹² In addition, we examined the metabolites of serotonin, dopamine, and norepinephrine: 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), respectively. Serotonin neurons participate in the regulation of CRF,¹³ and our previous work also suggested VFD behavioral hyposensitivity to the serotonin agonist *m*-CPP.¹⁴ We examined levels of MHPG in CSF, in light of the important stimulatory role of CRF on the noradrenergic locus coeruleus,¹⁵ as well as our previous observations of behavioral sensitivity to the α_2 autoreceptor antagonist yohimbine in adult VFD subjects.¹⁴ We analyzed levels of HVA in CSF to explore if the documented CRF and dopamine interaction found in rodents¹⁶ was evident in VFD-reared animals.

RESULTS

REARING EFFECTS

Levels of SOM, 5-HIAA, and HVA, but not MHPG, were each significantly elevated in VFD as compared with non-VFD subjects (Table). Analyses of the control group scores for SOM, 5-HIAA, HVA, and MHPG revealed no significant age effects, sex effects, or sex \times age effects. Levels of HVA showed the strongest, but nonsignificant, relationship with age (Pearson $r = -0.32$, $df = 27$; $P = .1$).

Because of the study design, it was difficult to determine if the changes observed in the VFD-reared subjects were due to the specific nature of maternal foraging demand and/or the joystick task. We therefore compared by 1-way ANOVA the 3 VFD-reared groups based on maternal foraging demand (digging with mesh, video task with mesh, and video task without mesh). There were no significant differences between the 3 groups for all parameters, including CRF and cortisol. We then compared only the offspring of the 4 mothers who were assigned to the variable digging paradigm with the non-VFD group. Levels of CRF ($t = 3$; $df = 16$; $P = .009$) and 5-HIAA ($t = 2.4$; $df = 10$; $P = .04$) were elevated, while cortisol levels were decreased ($t = 2.7$; $df = 17$; $P < .02$) in the digging VFD group in com-

were slowly withdrawn and stored at -70°C until analysis. All CSF sampling was performed between 10:15 AM and 11:30 AM to avoid diurnal confounds. Several hundred taps using this dosage of ketamine have been carried out without any evidence of short- or long-term difficulty. Animals were observed until they were fully recovered following the tap and did not appear to be in pain or distress during or after the procedure.

Levels of SOM in CSF were determined using radioimmunoassay techniques described in detail by Nemeroff and colleagues¹⁷ from the same CSF sample from which CRF was obtained. A second CSF sample was obtained under identical conditions several weeks later, from which CSF cortisol measures were taken,⁸ and provided CSF for 5-HIAA, HVA, and MHPG assays by the Department of Analytic Psychopharmacology, New York State Psychiatric Institute, New York City.

The 5-HIAA and HVA levels were determined using gas chromatography linked to a mass spectrometer according to the method described by Fri et al.¹⁸ The assay is routinely performed using a gas chromatograph/mass spectrometer with a direct capillary inlet with simultaneous ion monitoring in the electron impact mode. Within- and between-run coefficients of variation were less than 5% and less than 7% for each compound.

Cerebrospinal fluid MHPG was measured by gas chromatography/mass spectrometry operated in the simultaneous ion monitoring mode using deuterated MHPG as an internal standard. The method is essentially the same as that of Jimerson et al.¹⁹ Within- and between-run coefficients of variation were 4.6% and 5.1%, respectively. Laboratory personnel conducting the biochemical assays were blind to the subjects' rearing status.

For all CSF measures assayed, the LFD group scores were statistically indistinguishable from those of the HFD

group (the highest P value obtained comparing them was for 5-HIAA ($t=0.9$; $df=8$; $P=.4$) and so the LFD and HFD scores were combined into a single non-VFD group of 15 subjects for all subsequent comparisons. The occurrence of taps that were bloody or of insufficient quantity meant that the full complement of values was not available for each measure (available sample sizes for each parameter are indicated in the **Table**). To avoid undue influence produced by extreme outliers, it was decided to exclude from analysis any subject's data with a value of 2 SD from the group mean. Using this cutoff, only 3 (3%) of 106 total observations fulfill outlier status. Following assessment of experimental rearing effects, we determined the potential role of age at testing through the use of the age control group. A previously employed age control analysis⁸ was to be performed on the differentially reared groups if (1) a significant Pearson correlation was evident between age and the CSF parameter or (2) analysis of variance (ANOVA) revealed age, sex, or age \times sex effects.

Pearson correlations between neurochemical parameters and CRF and cortisol measures, which have been separately reported,⁸ were performed in the differentially reared groups. Group comparisons of correlations used the Fisher r -to- z transformation. Outlier scores on any particular measure may represent measurement artifacts, selective anomalies, or abnormalities that are reflected across more than one neurochemical system. To assess the latter possibility, and in contrast to the group analysis, individual outliers were included in the correlational analyses. When, however, examination revealed undue influence of any particular outlier score(s), the nonparametric Spearman correlation coefficient was used to determine the final significance. The control (ad libitum) group was analyzed similarly.

Significance level was set at an α value of $P<.05$, 2 tailed.

Mean Concentrations in Cerebrospinal Fluid (CSF) Somatostatin (SOM) and Biogenic Amines in Differently Reared Primate Groups*

CSF Measure	Primate Groups				t Test	df	P
	VFD		Non-VFD				
	Mean (SD)	No.	Mean (SD)	No.			
SOM, pg/mL	75.3 (30.0)	11	46.8 (29.0)	13†	2.4	22	.03
5-HIAA, ng/mL	71.7 (9.6)	14†	58.9 (6.8)	10†	3.6	22	.002
HVA, ng/mL	388.9 (68.7)	15	327.3 (44.5)	15	2.9	28	.007
MHPG, ng/mL	28.6 (4.4)	15	28.6 (2.7)	11	0	24	.98

* Infant primates were nursed by mothers randomly assigned to variable foraging demand (VFD) or nonvariable foraging conditions (Non-VFD). Concentrations of serotonin, dopamine, and norepinephrine metabolites (5-hydroxyindoleacetic acid [5-HIAA], homovanillic acid [HVA], and 3-methoxy-4-hydroxyphenylethylglycol [MHPG], respectively) and of SOM in CSF were determined.

† Outlier excluded.

parison with the non-VFD group. Thus, even when subjects who were video-reared are excluded, abnormal biochemistry in the VFD group is observed.

CORRELATIONS WITH CRF IN CSF

Strong positive correlations of CRF and SOM ($r=0.74$; $df=10$; $P=.009$), 5-HIAA ($r=0.72$; $df=14$; $P=.002$), and HVA

($r=0.80$; $df=14$; $P<.001$) were recorded for the VFD group. One VFD outlier subject who demonstrated unusually low 5-HIAA levels had correspondingly low CRF values. Follow-up Spearman rank order correlational analysis revealed that this outlier did not account for the CRF and 5-HIAA relationship in the VFD group ($t=2.7$; $r=0.6$; $P<.02$).

In the non-VFD group, the overall Pearson correlation of CRF and SOM was significant ($r=0.65$; $df=13$;

$P=.01$) but appeared dependent on an outlier subject who showed high levels on both measures. Spearman rank order correlation for this relationship was not significant ($n=14$; $t=1.3$; $P=.23$). There was no significant relationship between CRF levels and measures of either 5-HIAA or HVA in the non-VFD group ($r=-0.17$; $df=10$; $P=.6$; and $r=-0.04$; $df=14$; $P=.9$, respectively). The Fisher r -to- z transformation test revealed that the correlations between CRF and both 5-HIAA and HVA levels were significantly stronger in VFD than in non-VFD subjects ($\chi^2=5.2$; $df=1$; $P<.02$; and $\chi^2=7.8$; $df=1$; $P<.006$, respectively). Because heterogeneity of variance could have accounted for the group correlational differences, the Levene homogeneity of variance test was performed and showed none of the differentially reared groups' parameters to exhibit significant differences in variances in comparison with non-VFD subjects. No significant correlations involving CRF in the ad libitum-reared control group were observed.

CORRELATIONS EXCLUDING CRF IN CSF

In the VFD group, HVA correlated with 5-HIAA ($r=0.63$; $df=14$; $P=.01$) and MHPG ($r=0.57$; $df=14$; $P<.03$); in the non-VFD group, HVA correlated with MHPG. Unlike CRF, CSF cortisol did correlate with MHPG in both the VFD ($r=0.70$; $df=14$; $P=.003$) and non-VFD ($r=0.63$; $df=10$; $P=.04$) rearing groups.

In the control group, 1 subject with outlier 5-HIAA levels and 1 subject with outlier SOM levels were excluded. Levels of HVA correlated with those of 5-HIAA ($r=0.4$; $df=26$; $P<.03$). A counterintuitive positive cortisol and SOM correlation ($r=0.4$; $df=26$; $P<.03$) was observed.

COMMENT

The current report extends previous findings of long-term neurobiological sequelae following disturbances of early rearing in the nonhuman primate.²⁰⁻²⁴ We previously reported elevated CRF and decreased cortisol concentrations in the CSF of a group of bonnet macaques raised under VFD conditions.⁸ The current study demonstrated elevations of SOM, 5-HIAA, and HVA in the same group of primates, in comparison with predictably reared counterparts. No group differences for MHPG levels were noted. The lack of significant age effects in a separate control cohort suggests that the SOM, HVA, or 5-HIAA group differences were not likely due to age artifact. Nevertheless, the lack of an age-matched control group and the lack of homogeneity of the mode of VFD-rearing limits the generalizability of the findings.

Elevated levels of SOM, HVA, and 5-HIAA are positively correlated with previously reported CRF elevations.⁸ It is worth noting that the average relationships between measures also appeared to hold for the 3 "outlier" subjects, who generally showed extreme scores on more than 1 defining variable. Levels of MHPG correlated positively with levels of cortisol in the CSF of both groups, but did not correlate with CRF levels, consistent with the documented functional links between the noradrenergic system and the hypothalamic-pituitary-

adrenal axis²⁵ and also supporting the view that CRF in CSF is of extrahypothalamic origin.¹³ Although future cis-terial studies should include parallel blood sampling, we believe that CSF cortisol values are likely reflective of adrenal secretory activity. In both primates²⁶ and humans,²⁷ steroids, unlike peptides, equilibrate rapidly across the blood-brain barrier.

The complex and ostensibly coordinated chemical alterations observed in the VFD may be CRF-driven and have resulted from disturbance of optimal maternal-infant interaction. Another untested possibility is the excessive passage of glucocorticoids to the infant when lactation occurs during periods of stress.²⁸ Further developmentally adjusted, strategic behavioral and pharmacological interventions involving specific neurochemical systems are warranted.

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