Original Article

CHRONIC STRESS

Chronic Stress
Volume 2: 1–11
© The Author(s) 2018
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/2470547018785625
journals.sagepub.com/home/css

SAGE

Maternal Cerebrospinal Fluid Glutamate in Response to Variable Foraging Demand: Relationship to Cerebrospinal Fluid Serotonin Metabolites in Grown Offspring

Jeremy D. Coplan[1], Nishant K. Gupta[2], Sarah K. Flynn[2], Wade J. Reiner[2], David Gaita[2], Sasha L. Fulton[3], Anna V. Rozenboym[4], Jean E. Tang[3], Thomas B. Cooper[5], and J. John Mann[6,7]

Abstract

Background: Maternal response to allostatic overload during infant rearing may alter neurobiological measures in grown offspring, potentially increasing susceptibility to mood and anxiety disorders. We examined maternal cerebrospinal fluid (CSF) glutamate response during exposure to variable foraging demand (VFD), a bonnet macaque model of allostatic overload, testing whether activation relative to baseline predicted concomitant CSF elevations of the stress neuropeptide, corticotropin-releasing factor. We investigated whether VFD-induced activation of maternal CSF glutamate affects maternal–infant attachment patterns and offspring CSF 5-hydroxyindoleacetic acid concentrations.

Methods: Mother–infant dyads were exposed to the "VFD stressor," a paradigm in which mothers experience 16 weeks of foraging uncertainty while rearing their infant offspring. Through staggering the infant age of VFD onset, both a cross-sectional design and a longitudinal design were used. Maternal CSF glutamate and glutamine concentrations post-VFD exposure were cross-sectionally compared to maternal VFD naive controls. Proportional change in concentrations of maternal glutamate (and glutamine), a longitudinal measure, was evaluated in relation to VFD-induced elevations of CSF corticotropin-releasing factor. The former measure was related to maternal–infant proximity scores obtained during the final phases of VFD exposure. Maternal glutamatergic response to VFD exposure was used as a predictor variable for young adolescent offspring CSF metabolites of serotonin, dopamine, and norepinephrine.

Results: Following VFD exposure, maternal CSF glutamate concentrations correlated positively with maternal CSF CRF concentrations. Activation relative to baseline of maternal CSF glutamate concentrations following VFD exposure correlated directly with a) increased maternal-infant proximity during the final phases of VFD and b) offspring CSF concentrations of monoamine metabolites including 5-hydroxyindoleacetic acid, which was elevated relative to controls.

Conclusions: Activation of maternal CSF glutamate in response to VFD-induced allostasis is directly associated with elevations of maternal CSF corticotropin-releasing factor. Maternal CSF glutamate alterations induced by VFD potentially compromise serotonin neurotransmission in grown offspring, conceivably modeling human vulnerability to treatment-resistant mood and anxiety disorders.

[1]Department of Psychiatry and Behavioral Sciences, State University of New York Downstate Medical Center, Brooklyn, NY, USA
[2]College of Medicine, State University of New York Downstate Medical Center, Brooklyn, NY, USA
[3]Department of Psychiatry, College of Physicians and Surgeons, Columbia University Medical Center, New York State Psychiatric Institute, New York, NY, USA
[4]Department of Biological Sciences, Kingsborough Community College, City University of New York, Brooklyn, NY, USA
[5]Department of Psychopharmacology, Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY, USA
[6]Department of Psychiatry, Columbia University, New York, NY, USA
[7]Division of Molecular Imaging & Neuropathology, New York State Psychiatric Institute, New York, NY, USA

Corresponding author:
Jeremy D. Coplan, State University of New York Downstate Medical Center, 450 Clarkson Ave Brooklyn, New York, NY 11203, USA.
Email: copstat00@aol.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-Non-Commercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

Keywords
early life stress, variable foraging demand, nonhuman primates, glutamate, serotonin, corticotropin-releasing factor

Received 10 November 2017; Accepted 29 May 2018

Introduction

A central role for the excitatory amino acid neurotransmitter glutamate in the pathophysiology of mood and anxiety disorders has been well established.[1] The glutamate hypothesis has received support through the rapid, although transient, therapeutic effects of the N-methyl-D-aspartate antagonist, ketamine, in treatment-resistant depression[2,3] and the anxiolytic effects of antiglutamatergic agents, such as riluzole.[4,5] Lamotrigine, an anticonvulsant that blocks presynaptic release of glutamate,[6] plays an important role in prophylaxis of mood episodes in patients with bipolar type I disorders[7] although its efficacy in acute bipolar depression has not been supported.[8] Given that each of these medications entails modulation of glutamatergic neurotransmission, the preclinical effects of stress-induced alterations of cerebrospinal fluid (CSF) glutamate may shed light on mechanisms of certain therapeutic agents as well as the pathophysiology of mood and anxiety disorders.

Early life stress in the form of maternal loss or neglect has been cited as an important precedent to human mood and anxiety disorders.[9] Maternal unavailability can be modeled in nonhuman primates in the form of maternal variable foraging demand (VFD), a paradigm in which experimentally imposed maternal foraging uncertainty interrupts a normative maternal repertoire,[10] thereby inducing long-term biobehavioral sequelae in her offspring.[11] Maternal uncertainty of food perception is generated through alternating 2 week periods of low foraging demand (LFD) and high foraging demand (HFD) for 16 weeks during infant rearing.[12] We have reported, using proton magnetic resonance spectroscopy, that VFD rearing leads to a persistent hyperglutamatergic (Glx) spectral signal in anterior cingulate cortex with commensurate neuronal compromise as reflected by reduced N-acetylaspartate.[13] In an a priori hypothesis posed in 2001, stress-induced overactivity of glutamate-releasing neurons detected through serial cisternal CSF taps would conceivably be associated with a parallel activation of corticotropin-releasing factor (CRF), a key central stress neuropeptide[14] (see Supplement to Intro (SI1)).

Demonstration that activation relative to baseline of maternal CSF glutamate directly correlates with the elevated maternal CSF CRF concentrations observed following VFD exposure would support the view that the maternal CSF glutamate response observed following VFD tracks a key marker of allostatic overload, namely, CRF.[15,16] Further indirectly linking CSF glutamate activation to CRF increases can be tested through examination of maternal CSF glutamine changes following maternal VFD exposure. Glial-derived glutamine stores provide a direct pool for glutamate synthesis.[17] Thus, conceivably, the extent of depletion of maternal CSF glutamine may reflect, in part, the extent of glutamate synthesis[14] and, by proxy, inversely predict maternal CSF CRF response to VFD exposure. Relevance of maternal glutamatergic activation, moreover, would entail documentation of attachment behavior including variation of maternal–infant proximity.

Persistent elevations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in VFD cisternal CSF in comparison to non-VFD controls is a finding now observed in three separate VFD-reared nonhuman primate cohorts.[18–20] Valentino and coworkers[21] demonstrated that increasing glutamatergic input into the dorsal raphe nucleus initially increased serotonin neuronal firing, thereby increasing periraphe serotonin, ultimately activation somatodendritic 5-$HT1_A$ autoreceptors, thereby reducing serotonin neuronal firing. The latter scenario appears to compromise activation of postsynaptic serotonin receptors at distal target sites, particularly to hippocampus[20] and medial prefrontal cortex.[22] Rodent studies reveal that stimulation, either by electrical activation or by activation of 5-$HT2_A$ receptors located on pyramidal neurons in layer V of the medial prefrontal cortical, enlists glutamate-releasing dorsal raphe nucleus afferents[23] (see SI2).

In the current study, the investigators examined (1) the effects of a form of maternal allostatic overload[24]—maternal VFD—on maternal CSF glutamate and glutamine concentrations, (2) whether maternal CSF glutamate activation following VFD exposure directly correlates with maternal CSF CRF concentrations elevations while also predicting maternal–infant proximity patterns, and (3) whether relative activation of maternal CSF glutamate may, in part, through a previously documented, yet poorly elucidated, process of ''biological synchronization,''[25] exhibit the potential for ''transgenerational'' transmission of altered neurobiology from mother to grown offspring, ultimately impacting adolescent serotonergic systems.

Materials and Methods

Subjects

Eighteen mother–infant dyads of bonnet macaques (*Macaca radiate*) were studied in both a *longitudinal* (all VFD exposed) and *cross-sectional* design[26] of "early" VFD-exposed (nine) versus "late" VFD-exposed (nine) dyads examined prior to VFD exposure (see Figure 1). For the within-subject longitudinal analysis, 14 maternal–infant VFD-exposed dyads (7 male and 7 female infants) on whom pre- and postmaternal VFD CSF glutamate/glutamine concentrations and maternal–infant dyadic proximity data were available. Of these 14 infants, 8 as young adolescent offspring (3 male and 5 females) had CSF monoamine concentrations available for analysis. These eight VFD offspring were compared to 14 unstressed historical controls[20] of comparable age, weight, and sex distribution. This latter comparison would serve to situate the range of CSF 5-HIAA values observed in the eight VFD offspring as elevated. The subjects of the current report have been the focus of previous reports,[20,25,27,28] but maternal CSF glutamate and CSF glutamine concentrations in response to VFD have not been reported earlier (see Supplement to Methods (SM) 1).

VFD Rearing

Mother–infant dyads were group-housed in four pens of four to seven dyads each and stabilized for at least four weeks prior to VFD onset. Fathers in the VFD paradigm were routinely removed from the pen following female impregnation. After infants reached at least two months of age, dyads were subjected to a standard VFD procedure that involved eight alternating two-week blocks (=16 weeks) in which maternal food was either readily accessible (LFD) or more difficult to obtain (HFD).[11]

Foraging carts were utilized to control difficulty in obtaining food. In the HFD condition, mothers would reach through apertures in a foraging cart to dig through clean wood chip in order to obtain food rations.[11] Food deprivation is avoided in the HFD condition, illustrated by normal infant weight gain and maintenance of adult baseline weights throughout the procedure.[29] In the control (LFD) condition, mothers are able to pick up freely available food items.[11] After two years of age, VFD

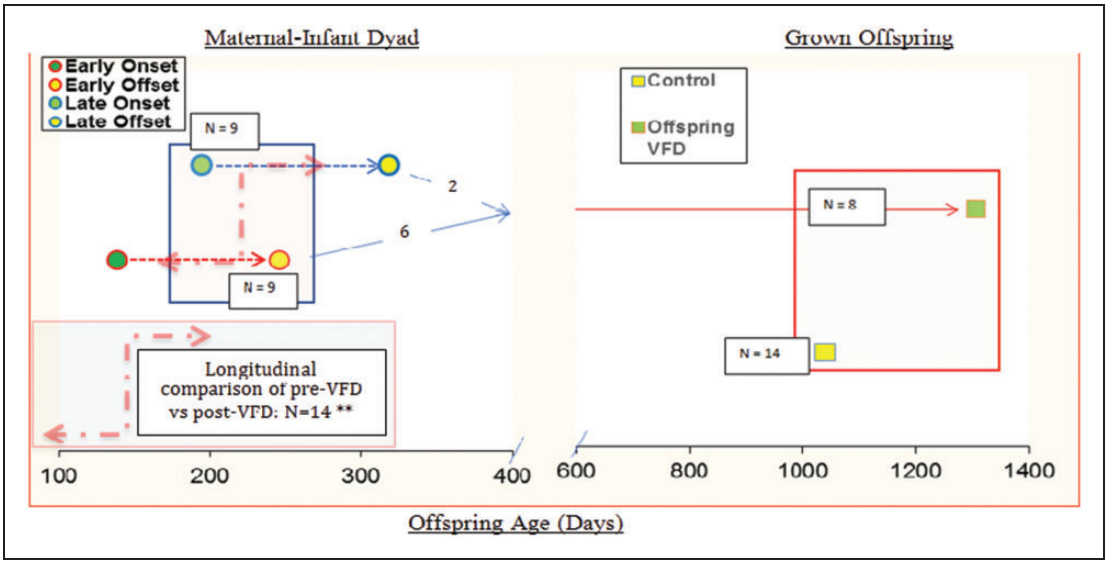

Figure 1. Flow chart of study design including a cross-sectional and longitudinal design for the study of a single VFD-exposed cohort. *Staggered onset experimental design based on infant age*: The blue-outlined red-shaded box in the left panel indicates where the early VFD group (early onset) enters into the end of VFD (early offset, N = 9) and the late VFD group (late onset, N = 9) initiates VFD to allow for a cross-sectional comparison (total N = 18). The red-outlined blue-shaded box in the bottom left corner of the left panel includes a double-arrowed dashed line that in the center of the left panel denotes that, along with the cross-sectional design, a longitudinal pre-VFD/post-VFD comparison (N = 14, paired data not available = 4) can be made. In the right panel, the red-outlined box indicates data on eight VFD offspring available for (1) longitudinal relationship to maternal–infant data and (2) cross-sectional comparison to 14 non-VFD historical controls. *X-axis: infant ages (days)*: early onset (mean (SD) = 138.50 (13.44 days)); early offset (246.00 (16.97) (N = 9)); late onset (194.83 (20.99) (N = 9)); late offset (318.83 (18.65)); right panel: longitudinal studies: offspring VFD (1306.70 days, N = 8 (two from early and six from late VFD reared); historical controls (1040.25 days, N = 14)). **Paired data not available four subjects.
VFD: variable foraging demand.

offspring were separated from their mothers and housed in VFD-reared peer social groups. Non-VFD subjects, that is, historical controls, were always housed with non-VFD-reared peers.

CSF Sampling

Both maternal and infant cisternal CSF samples were obtained prior to the onset of VFD and during the last week of the final HFD cycle (see SM2).

For grown offspring, subjects were socially housed in their natal peer groups, and a young adolescent CSF sample was obtained[28] in the same manner as described earlier. Detailed data relating to time from capture to ketamine injection or ketamine injection to specimen retrieval are not available.

CSF Glutamate/Glutamine Measurements

As described earlier, maternal CSF samples were obtained prior to and following the 16 weeks of VFD exposure. Glutamate in CSF was determined by a modified liquid chromatographic procedure with automated precolumn derivatization[30] (see SM3).

CSF CRF Measurements

CRF concentrations in maternal CSF were measured by a well-characterized radioimmunoassay as described earlier[31] (see SM4).

Behavioral Observations

Maternal–Infant Proximity (Dyadic Distance): During daily behavioral sessions, five days per week, each mother–infant dyad was observed for 20 s in a random sequence on five separate occasions. Dyadic distance was scored during each observation on a scale of 1–5 (1 describing the greatest proximity and 5 describing the least) (see SM5 for details). Mother–infant dyadic distance was aggregated for the final LFD 2-week period and then for the final HFD 2-week period during the final 4 weeks of the 16-week VFD cycle. The LFD–HFD difference in dyadic proximity scores was determined.

Monoamine Measurements

CSF monoamine assays[20] were performed on 8 subjects (3 males and 5 females) at a mean age of 3.58 years (corresponding approximately to human early adolescence) and compared to 14 subjects from a historical control group (2.85 years) (10 males and 4 females). CSF samples were used to measure concentrations of the central nervous system (CNS) metabolites of serotonin (5-HIAA), norepinephrine (3-methoxy-4-hydroxyphenylglycol (MHPG)), and dopamine (homovanillic acid (HVA)). The CSF monoamine metabolites were assayed using high-performance liquid chromatography with an electrochemical detector described in detail elsewhere.[32]

Statistical Analyses

Normality of distribution of maternal CSF glutamate concentrations and maternal CSF glutamine concentrations was performed using the Kolmogorov–Smirnov (KS) test, and data were inspected for outliers. A combination of cross-sectional and longitudinal analyses was performed in a single cohort.[26] The first component of the analyses performed a cross-sectional examination of VFD versus VFD naive dyads. This comparison was achieved by examining "early VFD" exposure dyads[27] at the end of their VFD exposure compared to "late VFD"-reared subjects[27] prior to VFD exposure (i.e., naive) conditions (see Figure 1). Infant sex distribution between-groups was compared using 2×2 tables and chi-square statistics. Imbalance for sex distribution, if noted, led to sex being used as a covariate. Student t tests were used to examine for group differences for maternal age, maternal body mass, infant age, and infant weight for use as covariates should significant group effects be detected. Maternal CSF glutamate and glutamine concentrations were also compared between the two groups. A general linear model (GLM) was used to examine the predictive effect of maternal CSF glutamate concentrations on maternal CSF CRF concentrations while using VFD exposure versus nonexposure as a categorical variable. The interaction term of VFD exposure $\times$ maternal CSF glutamate concentrations would interrogate whether glutamate played a predictive role in determining CSF CRF concentrations in VFD-exposed mothers which was distinguishable from VFD naive mothers. GLM was followed up by within-group Pearson's correlations to examine for glutamate/CRF associations.

The second component of the statistical analysis was a longitudinal examination of VFD-exposed dyads only and investigated the relationship between proportional change (Δ) ((post-VFD $-$ pre-VFD)/pre-VFD) maternal CSF glutamate and glutamine concentrations as predictor variables in response to VFD exposure. A proportional Δ value was used since the value alone (post-VFD $-$ pre-VFD) lacks integration of baseline values by which to accurately judge the magnitude of the Δ effect. The within-subject longitudinal analysis examined the relationship between proportional Δ maternal CSF glutamate concentrations to maternal–infant proximity scores from (a) the final LFD phase (week 13–14), (b) the final HFD phase (week 15–16), and (c) the final Δ maternal–infant proximity scores (HFD minus LFD).

A within-subject analysis examined the relationship between proportional Δ maternal CSF glutamate concentrations and young adolescent offspring CSF monoamine metabolite concentrations integrated into the model as repeated dependent measures (5-HIAA, HVA, and MHPG), followed by univariate analyses. Post hoc Pearson's correlations were performed for significant GLMs to clarify directionality of effects (see SM6, Analysis of Confounds Including Heritability). Probability was set at $p \leq 0.05$, two-tailed.

Results

Cross-Sectional Analysis of VFD-Exposed Versus Nonexposed Dyads

Of the 18 mother–infant dyads examined, 9 had been exposed to the VFD paradigm (5 males, 4 females), whereas the remaining subjects served as VFD naive controls (2 males, 7 females) (sex distribution 2×2 tables: χ^2 ($df = 1$) = 2.10; $p = 0.15$). Distribution of maternal CSF glutamate concentrations (KS, $d = 0.28$, $p < 0.10$), maternal CSF glutamine concentrations (KS, $d = 0.16$, $p > 0.20$), and maternal CSF CRF (KS, $d = 0.086$, $p > 0.20$) were considered normal. No outliers were noted. There were no cross-sectional group differences for maternal age, maternal weight, infant age, or infant weight (Table 1). Nor did the latter four independent variables correlate with CSF glutamate or CSF glutamine concentrations. However, since age effects for mothers and infants were near significant, each was used as covariates.

Using GLM (Figure 2), a group × post-VFD maternal CSF glutamate concentration interactive effect was noted in the prediction of post-VFD maternal CSF CRF concentrations ($F_{(1,14)} = 16.35$; $p = 0.001$). A positive glutamate/CRF correlation was present in VFD-exposed mothers ($r = .82$, $N = 9$, $p = 0.006$) but not in VFD naive mothers ($r = -.05$; $N = 9$, $p = .88$) (see Figure 2) (see Supplement Results 1 (SR1) for control variable effects). Maternal CSF CRF concentrations (ng/ml) in post-VFD were increased in comparison to VFD naive subjects (VFD mean (standard error, SE) = 460.62 (34.40) vs. VFD naive mean (SE) = 359.82 (34.40); $F_{(1,14)} = 10.92$; $p = 0.005$) when controlling for CSF glutamate. No group differences were found in the cross-sectional analysis for maternal CSF glutamate concentrations or maternal CSF glutamine concentrations (Table 1).

Within-Subject Glutamate and Glutamine Analyses: Prediction of Maternal and Offspring Neurobiology

Fourteen maternal–infant dyads (7 male and 7 female infants) exposed to VFD had both pre- and postmaternal CSF glutamate concentrations and maternal CSF glutamine concentrations assayed. No change was noted between pre- versus post-VFD maternal glutamate concentrations (pre-VFD mean (standard deviation, SD) = 1.18 (0.68) ng/ml vs. post-VFD mean (SD) = 1.19 (0.49); t value = −0.03; $df = 13$, $p = 0.98$). Maternal CSF glutamate concentration change was therefore negligible (mean (SD) = 0.007 (0.9) ng/ml ($N = 14$)) despite a wide range of post- minus pre-VFD Δ values (−2.1 to 1.8) (see SR2 for "Further Analyses Maternal CSF Glutamate Concentrations" and ratio analyses).

Maternal CSF Glutamine Concentrations

Using a GLM, proportional Δ CSF glutamine concentrations inversely predicted Δ maternal CSF CRF (ng/ml) concentrations (mean (SD) = 86.52 (121.00) ng/ml; $N = 13$; $F_{(1,12)} = 9.26$; $p = 0.01$; (Pearson's $r = -.70$, $N = 13$, $p = 0.008$)) (Figure 3). Proportional Δ CSF glutamine concentrations did not predict pre-VFD (mean (SD) = 359.05 (114.83) ng/ml; $N = 13$) or post-VFD maternal CSF CRF concentrations (mean (SD) = 434.67 (140.57) ng/ml; $N = 13$). No infant sex or paternity effects were observed (Supplement Table 2 and SR1).

There was no significant change in CSF glutamine concentrations (pre-VFD mean (SD) = 539.76 (47.65)

Table 1. Comparison of maternal independent and dependent variables in VFD-exposed versus unexposed dyads.

	Non-VFD	VFD			
	Mean ± SD	Mean ± SD			
Variable	(N = 9)	(N = 9)	t	df	p
CSF glutamate (ng/ml)	1.21 ± 0.86	1.2 ± 0.27	0.04	16	0.97
CSF glutamine (ng/ml)	542.53 ± 50.9	527.19 ± 44.89	0.68	16	0.51
Mother weight (kg)	4.9 ± 0.95	5.16 ± 1.14	−0.51	16	0.62
Mother age (years)	9.4 ± 3.8	7.2 ± 2.1	1.56	16	0.14
Infant age (days)	200 ± 25 (N = 8)	231 ± 45	1.71	15	0.11
Infant weight (kg)	1.38 ± 0.24	1.31 ± 0.30 (N = 8)	1.31	15	0.59

Note: CSF: cerebrospinal fluid; SD: standard deviation; VFD: variable foraging demand.

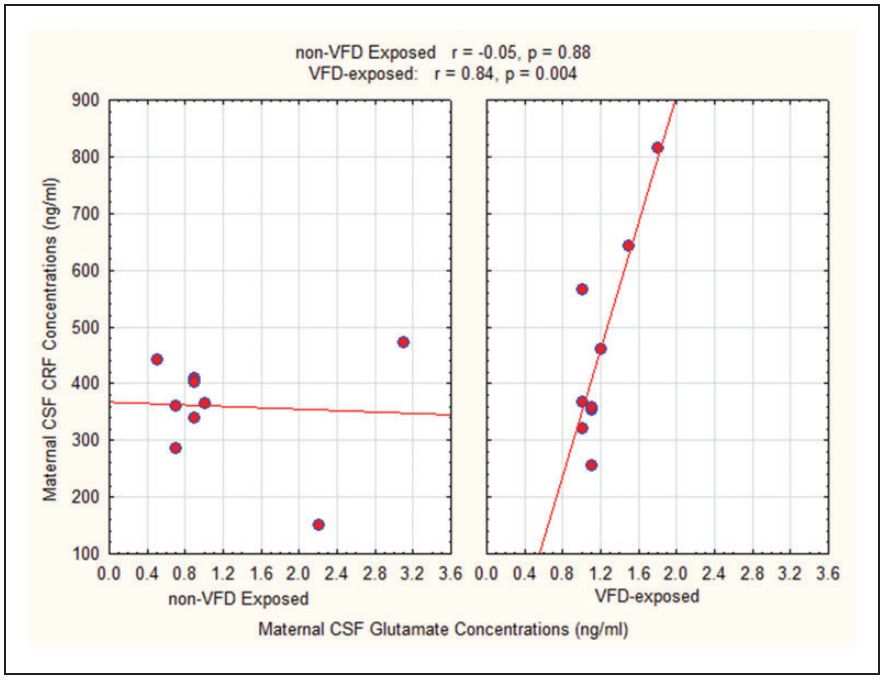

Figure 2. Relationship between maternal CSF glutamate concentrations and maternal CSF CRF concentrations as a function of maternal VFD exposure. Using a general linear model, there was a rearing group × CSF glutamate concentration interactive effect ($F_{(1,14)} = 16.35$; $p = 0.001$) a which stemmed from positive glutamate/CRF correlation in VFD-exposed mothers that was significantly distinguishable from the corresponding nonsignificant correlation in non-VFD-exposed mothers.
CSF: cerebrospinal fluid; CRF: corticotropin-releasing factor; VFD: variable foraging demand.

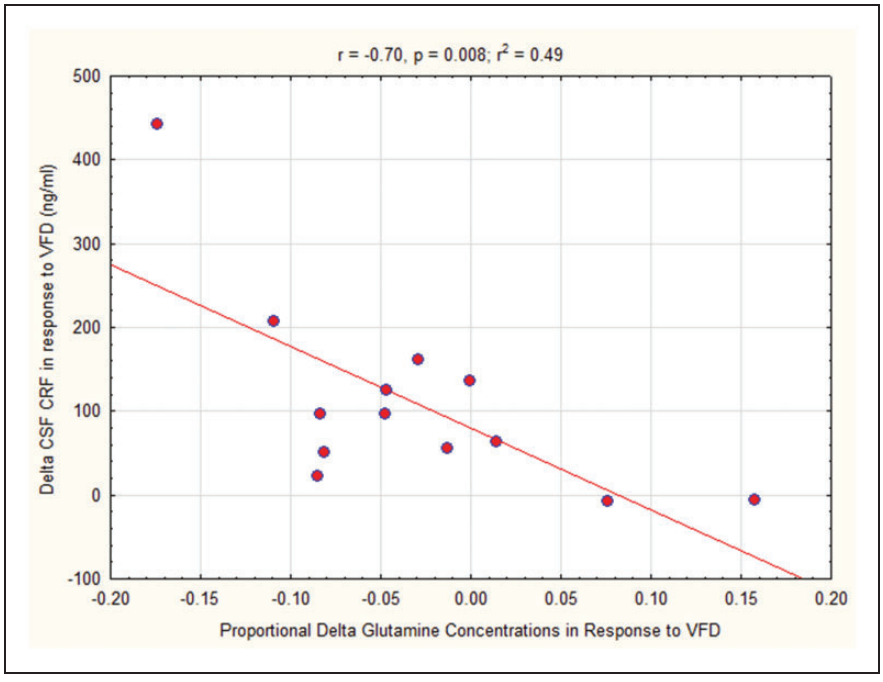

Figure 3. Maternal ΔCSF CRF (pg/ml) in response to VFD plotted against maternal proportional ΔCSF glutamine in response to VFD. Relative reductions in CSF glutamine are associated with CSF CRF increases.
CSF: cerebrospinal fluid; CRF: corticotropin-releasing factor; VFD: variable foraging demand.

ng/ml vs. post-VFD mean (SD) = 521.22 (51.59); t value = 1.61; df = 13, p = 0.13). Maternal CSF glutamine concentration change was, however, discernable (mean (SD) was −18.54 (43.06) ng/ml (N = 14)).

Dyadic Proximity

Proportional Δ maternal CSF glutamate concentrations did not predict maternal–infant dyadic proximity during either the final two weeks LFD (week 13–14) (mean (SD) = 3.31 (0.80) score N = 13) or the subsequent final two week HFD (week 15–16) (mean (SD) = 3.50 (1.02) score; N = 13; paired t = 0.80; df = 12; p = 0.43) phases, but the variable did positively predict the dyadic proximity difference (HFD minus LFD) (mean (SD) = −0.19 (0.83) score; N = 13; $F_{(1,11)} = 14.07$; p = 0.003; partial $\eta^2 = 0.56$). Inspection of Figure 4 (r = −.75; N = 13; p = 0.003) indicates that those mothers who exhibited proportional increases in CSF glutamate concentrations in response to VFD exposure showed an increase in dyadic proximity when transitioning from the final LFD to final HFD phase. Conversely, proportional decreases in maternal CSF glutamate concentrations in response to VFD exposure were associated with dyadic distancing from final LFD to HFD (see SR3 for control variables).

Offspring CSF Monoamines Metabolite Concentrations

CSF monoamine studies (N = 8; 3 males, 5 females) were done at a mean (SD) age of 3.58 (0.31) years at a mean weight (SD) of 4.44 (0.34) kg corresponding approximately to young adolescence in humans—all subjects had been exposed to VFD rearing. Because CSF 5-HIAA correlated directly with CSF HVA (n = 8; r = .97; p = 0.001) and CSF MHPG (n = 8; r = .75; p = 0.03) and CSF HVA correlated with CSF MHPG (n = 8; r = .72; p = 0.04), these three variables were used as repeated dependent measures in a GLM.

Proportional maternal Δ CSF glutamate positively predicted offspring CSF monoamine metabolites ($F_{(1,6)} = 16.63$; p = 0.007; partial $\eta^2 = 0.73$; 5 factors > large effect size) with a repeated measures × proportional maternal ΔCSF glutamate concentration interactive effect noted ($F_{(2,12)} = 12.08$; p = 0.001). Univariate analyses revealed that proportional Δ maternal CSF glutamate concentrations in response to VFD exposure directly predicted CSF 5-HIAA concentrations (ng/ml) ($F_{(1,6)} = 16.31$; p = 0.007; r = .86; N = 8; p = 0.007), offspring CSF HVA concentrations (ng/ml) ($F_{(1,6)} = 14.21$; p = 0.009; r = .84; N = 8; p = 0.009), and offspring CSF MHPG concentrations (ng/ml) ($F_{(1,6)} = 31.48$; p = 0.001; r = .92; N = 8; p = 0.001)—the

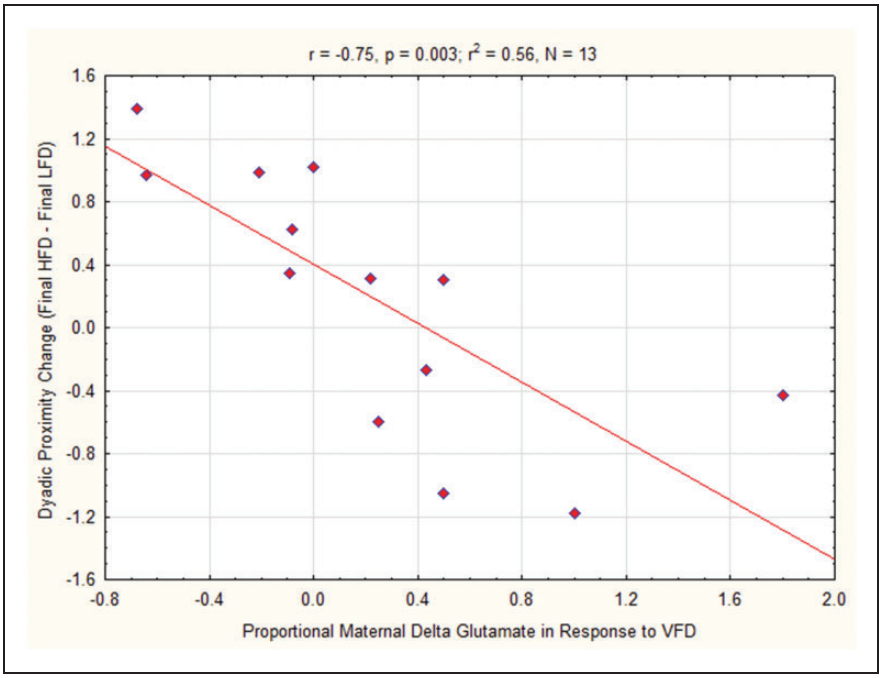

Figure 4. Relationship between proportional maternal CSF glutamate concentration change following VFD exposure and change in dyadic proximity (HFD minus LFD) from the final LFD to HFD phase of the VFD paradigm. Maternal proportional ΔCSF glutamate concentrations ((post-VFD minus pre-VFD)/pre-VFD) inversely predicted the scoring change in maternal–infant (dyadic) distance (HFD minus LFD) ($F_{(1,11)} = 14.07$; p = 0.008; r = −.75; N = 13; p = .0.003; partial $\eta^2 = 0.56$).
HFD: high foraging demand; LFD: low foraging demand; VFD: variable foraging demand.

relatively robust effect for MHPG in comparison to the two other two bivariate relationships accounting for a repeated measures interactive effect (see Supplement Table 5a and 5b and Supplement Figure 1 for paternity effects).

Confirming that VFD offspring CSF 5-HIAA concentrations were indeed increased, significant elevations in comparison to 14 historical controls were observed, when controlling for sex, age, and weight ($F_{(1,17)} = 8.17$; $p = 0.01$; partial $\eta^2 = 0.32$ (>two-fold a large effect size)) (Supplementary Table 6). Proportional Δ maternal CSF glutamine concentrations did not predict offspring CSF monoamine concentrations. No sex effects were noted. Controlling for either infant age or maternal age did not affect the results.

Potential outlier effects were addressed using the Spearman nonparametric correlation. Proportional Δ maternal CSF glutamate concentrations increases positively predicted offspring CSF 5-HIAA concentrations (Spearman $r = .94$; $N = 8$; $p = 0.0004$) (see Supplement Table 7). In addition, bivariate bootstrapping was performed using 1000 iterations (SPSS 24) (see Supplement Table 8), and 95 percentile confidence intervals remained significant.

Effect Sizes

a. The VFD exposure × maternal CSF glutamate concentration interaction effect size in the prediction of maternal CSF CRF concentrations—partial $\eta^2 = 0.53$ (0.14 = large effect size, three-fold > large effect size).
b. Proportional Δ maternal CSF glutamate concentration is inversely associated with final LFD to HFD differences in dyadic proximity—partial $\eta^2 = 0.49$ (three-fold > large effect size).
c. Proportional Δ maternal CSF glutamate concentration is directly associated with grown offspring CSF 5-HIAA concentrations—partial $\eta^2 = 0.62$ (four-fold > large effect size).

Discussion

The findings of the current study indicate, in cross-sectional analysis (Figure 1), that mothers rearing their infants while exposed to maternal allostatic overload in the form of VFD exposure exhibit maternal CSF glutamate concentrations that are directly related to the maternal CSF CRF concentrations, an effect not present in VFD naive mothers (Figure 2). In longitudinal analysis (Figure 1), VFD-induced elevations of maternal CSF CRF concentrations observed post-VFD are inversely related to the proportional Δ (= change) maternal CSF glutamine concentrations (Figure 3). The latter effect is consistent with the view that the glutamine pool, which serves as reservoir for glutamate synthesis,[1,14] could conceivably be depleted should glutamate be utilized in the expression of VFD-induced increases in maternal CSF CRF.[14] These data may provide evidence which indicates that one key marker of maternal allostatic overload, central CRF expression,[15] is associated with alterations in the maternal CSF glutamate measures in the expected direction (relative glutamate increase, relative glutamine decrease).

Moreover, we demonstrated that proportional Δ maternal CSF glutamate is tethered to dyadic behavioral response as the former measure predicts the difference scores in maternal–infant dyadic distance between the final HFD (last 2 weeks) minus final LFD (last 4 to 2 weeks) phase of the 16-week VFD paradigm. The data indicate that activation of maternal CSF glutamate relative to baseline is associated with increasing dyadic proximity when transitioning between the final LFD to HFD phase (and vice versa) (Figure 4). Dyadic proximity is a behavioral response that has been posited to reflect distressed attachment and dyadic vulnerability.[10,33]

Increased CSF glutamate relative to baseline in VFD-exposed mothers predicts persistent serotonin-related alterations in young adolescent offspring. The latter "transgenerational effect" is manifest as persistent increases in CSF 5-HIAA concentrations (Figure 5), a view that is validated through demonstration that VFD-reared offspring exhibit CSF 5-HIAA elevations in comparison to a historical control group (see Supplement to Discussion (SD) 2). Effects remain significant after controlling for sex, age, and weight of subjects (Figure 6, Supplement Table 5a and 5b). Taken together, maternal CSF glutamate measures in response to VFD are validated by the associations described earlier. "Transgenerational transmission," a term we have used previously in the same context[34] is observed, implying that environmentally induced phenotypic features observed in the mother may be observed in their infants and subsequently in their grown offspring. It is of note that VFD exposure did not increase *mean* maternal CSF glutamate concentrations when compared to the non-VFD-exposed controls despite the increase in CSF CRF concentrations (Table 1). Nor are mean maternal CSF glutamate concentrations post-VFD exposure distinguishable from their mean pre-VFD values (see SD1and SD3 for further details).

The CSF 5-HIAA measures in VFD-reared offspring are further validated by the expected high correlations observed between CSF 5-HIAA and other monoamine metabolites, CSF HVA (dopamine) and CSF MHPG (norepinephrine) (Supplement Table 4 for correlation matrix). Moreover, each offspring monoamine metabolite is separately predicted by proportional Δ maternal CSF

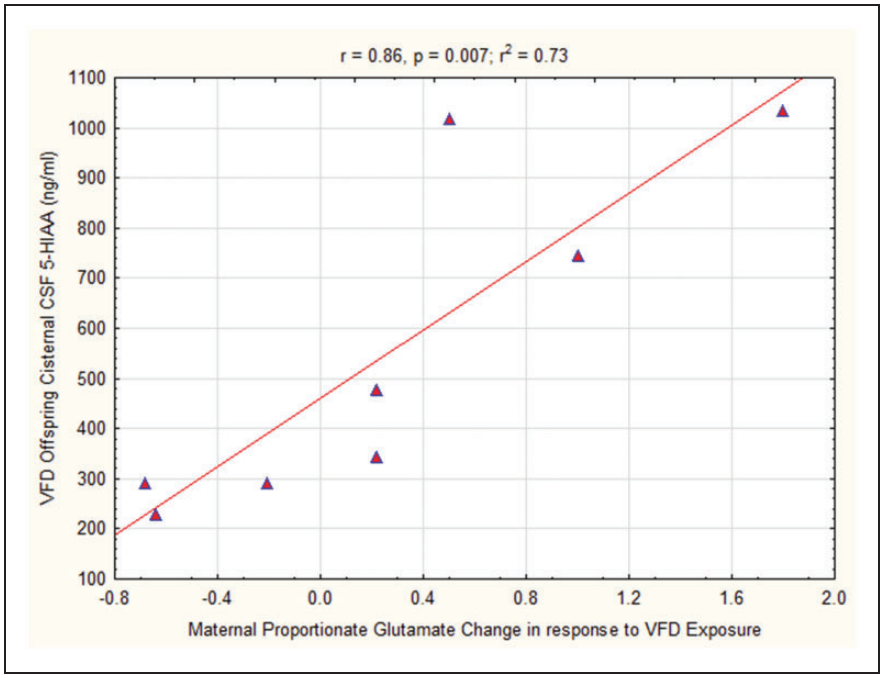

Figure 5. Relationship between maternal proportional ΔCSF glutamate concentrations in response to VFD exposure and offspring CSF 5-HIAA concentrations. Change in maternal proportional ΔCSF glutamate concentrations ((post-VFD minus pre-VFD)/pre-VFD) positively predicted offspring CSF 5-HIAA concentrations (ng/ml) (see text for Spearman correlations and bootstrapping method).
CSF: cerebrospinal fluid; 5-HIAA: 5-hydroxyindoleacetic acid; VFD: variable foraging demand.

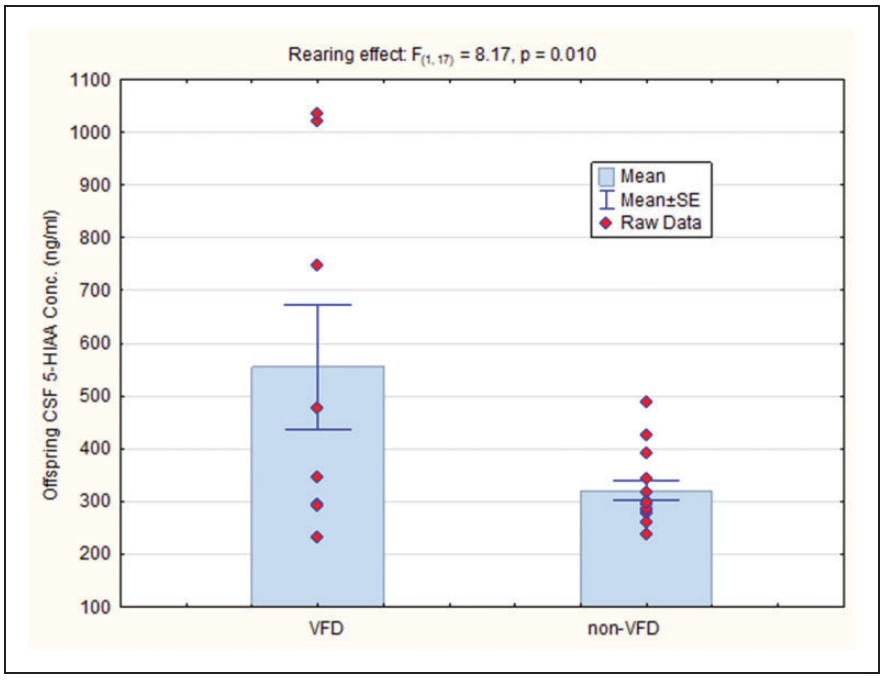

Figure 6. Scatterplot with SEs of CSF 5-HIAA concentrations in early adolescent VFD offspring versus non-VFD controls.
CSF: cerebrospinal fluid; 5-HIAA: 5-hydroxyindoleacetic acid; SE: standard error; VFD: variable foraging demand.

glutamate (Supplement Table 5a and 5b). Paternity or maternal effects each represent a potentially heritable influence that could conceivably confound the findings but appear to play, at most, a minimal role in influencing the transgenerational transmission results (see Supplement Figure 1).

Limitations of the current study include the nonspecificity of CSF glutamate/glutamine measures and restriction of the data to a relatively small sample size. In the current study, cisternal CSF, which is not specific to any regional source of glutamate, may conceivably preferentially reflect the peri-raphe milieu.[20] Although magnetic resonance spectroscopy in nonhuman primates offers a means for direct quantification of glutamatergic measures,[13] CSF analysis may provide an overall index of total brain glutamate/glutamine spillover into CSF. Also, CSF measures, it should be noted, do not provide a direct measure of metabolic/signaling specificity.[35] That mean maternal CSF glutamate concentrations appear to be controlled within a very narrow range despite 16 weeks of maternal VFD exposure suggests that cisternal CSF measures are not entirely nonspecific.

Another potential limitation of the study involves the lack of a control follow-up group. We were able, using a simultaneous cross-sectional and longitudinal single cohort design,[26] to demonstrate VFD-exposed versus VFD naive differences. Through the use of a historical control group, we were able to demonstrate that offspring VFD exhibit persistent *elevations* in CSF 5-HIAA concentrations. Although it is possible that transgenerational correlations may have also existed in a normal control group, the CSF 5-HIAA concentrations would not, in all likelihood, be elevated (see SD4 for lack of control for multiple testing).

The current study documents alterations within the glutamatergic system following maternal exposure to prolonged and cumulative stress in the VFD model—a putative example of allostatic overload—and thus bears relevance to certain mood and anxiety disorders.[2–5] We provided support for our hypothesis through demonstration that alterations of maternal CSF glutamate concentrations in response to VFD were associated with alterations in stress-related behavior and physiology of the mother, the mother–infant dyad, and grown offspring—the latter effect validating, in part, the notion of transgenerational transmission across discrete biological systems.[35] Further studies integrating epigenetic mechanisms into the area of transgenerational transmission are warranted.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institute of Mental Health (grant R01MH59990A; to J.D.C.).

ORCID iD

Nishant K. Gupta http://orcid.org/0000-0002-8006-3786
Sasha L. Fulton http://orcid.org/0000-0003-3626-7412
J. John Mann http://orcid.org/0000-0002-0265-6586

References

1. Sanacora G, Zarate CA Jr, Krystal J, Manji HK. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nat Rev Drug Discov* 2008; 7: 426.
2. Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 1997; 17: 2921–2927.
3. Price RB, Nock MK, Charney DS, Mathew SJ. Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol Psychiatry* 2009; 66: 522–526.
4. Mathew SJ, Amiel JM, Coplan JD, Fitterling HA, Sackeim HA, Gorman JM. Open-label trial of riluzole in generalized anxiety disorder. *Am J Psychiatry* 2005; 162: 2379–2381.
5. Zarate CA, Payne JL, Quiroz J, et al. An open-label trial of riluzole in patients with treatment-resistant major depression. *Am J Psychiatry* 2004; 161: 171–174.
6. Cunningham MO, Jones RS. The anticonvulsant, lamotrigine decreases spontaneous glutamate release but increases spontaneous GABA release in the rat entorhinal cortex in vitro. *Neuropharmacology* 2000; 39: 2139–2146.
7. Calabrese JR, Bowden CL, Sachs G, et al. A placebo-controlled 18-month trial of lamotrigine and lithium maintenance treatment in recently depressed patients with bipolar I disorder. *J Clin Psychiatry* 2003; 64: 1013–1024.
8. Calabrese JR, Bowden CL, Sachs GS, Ascher JA, Monaghan E, Rudd GD. A double-blind placebo-controlled study of lamotrigine monotherapy in outpatients with bipolar I depression. *J Clin Psychiatry* 1999; 60: 79–88.
9. Heim C, Shugart M, Craighead WE, Nemeroff CB. Neurobiological and psychiatric consequences of child abuse and neglect. *Dev Psychobiol* 2010; 52: 671–690.
10. Coplan JD, Karim A, Chandra P, Germain GS, Abdallah CG, Altemus M. Neurobiology of maternal stress: role of social rank and central oxytocin in hypothalamic–pituitary adrenal axis modulation. *Front Psychiatry* 2015; 6: 100.
11. Coplan JD, Andrews MW, Rosenblum LA, et al. 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. *Proc Natl Acad Sci USA* 1996; 93: 1619–1623.

12. Andrews MW, Rosenblum LA. The development of affiliative and agonistic social patterns in differentially reared monkeys. *Child Dev* 1994; 65: 1398–1404.
13. Mathew SJ, Shungu DC, Mao X, et al. A magnetic resonance spectroscopic imaging study of adult nonhuman primates exposed to early-life stressors. *Biol Psychiatry* 2003; 54: 727–735.
14. Mathew SJ, Coplan JD, Smith EL, Schoepp DD, Rosenblum LA, Gorman JM. Glutamate-hypothalamic-pituitary-adrenal axis interactions: implications for mood and anxiety disorders. *CNS Spectr* 2001; 6: 555–564.
15. Schulkin J, Gold PW, McEwen BS. Induction of corticotropin-releasing hormone gene expression by glucocorticoids: implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 1998; 23: 219–243.
16. Schulkin J, McEwen BS, Gold PW. Allostasis, amygdala, and anticipatory angst. *Neurosci Biobehav Rev* 1994; 18: 385–396.
17. Daikhin Y, Yudkoff M. Compartmentation of brain glutamate metabolism in neurons and glia. *J Nutr* 2000; 130: 1026S–1031S.
18. Coplan JD, Trost RC, Owens MJ, et al. Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch Gen Psychiatry* 1998; 55: 473–477.
19. Mathew SJ, Coplan JD, Smith EL, et al. 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.
20. Coplan JD, Fulton SL, Reiner W, et al. Elevated cerebrospinal fluid 5-hydroxyindoleacetic acid in macaques following early life stress and inverse association with hippocampal volume: preliminary implications for serotonin-related function in mood and anxiety disorders. *Front Behav Neurosci* 2014; 8: 440.
21. Commons KG, Connolley KR, Valentino RJ. A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacology* 2003; 28: 206.
22. Bach H, Huang YY, Underwood MD, Dwork AJ, Mann JJ, Arango V. Elevated serotonin and 5-HIAA in the brainstem and lower serotonin turnover in the prefrontal cortex of suicides. *Synapse* 2014; 68: 127–130.
23. Celada P, Puig MV, Casanovas JM, Guillazo G, Artigas F. Control of dorsal raphe serotonergic neurons by the medial prefrontal cortex: involvement of serotonin-1A, GABAA, and glutamate receptors. *J Neurosci* 2001; 21: 9917–9929.
24. McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. *Horm Behav* 2003; 43: 2–15.
25. Coplan JD, Altemus M, Mathew SJ, et al. Synchronized maternal-infant elevations of primate CSF CRF concentrations in response to variable foraging demand. *CNS Spectr* 2005; 10: 530–536.
26. Rowe JW, Andres R, Tobin JD, Norris AH, Shock NW. The effect of age on creatinine clearance in men: a cross-sectional and longitudinal study 2. *J Gerontol* 1976; 31: 155–163.
27. Coplan JD, Smith EL, Altemus M, et al. Maternal-infant response to variable foraging demand in nonhuman primates. *Ann N Y Acad Sci* 2006; 1071: 525–533.
28. Coplan JD, Abdallah CG, Kaufman J, et al. Early-life stress, corticotropin-releasing factor, and serotonin transporter gene: a pilot study. *Psychoneuroendocrinology* 2011; 36: 289–293.
29. Rosenblum LA, Paully GS. The effects of varying environmental demands on maternal and infant behavior. *Child Dev* 1984; 1: 305–314.
30. Spink DC, Swann JW, Snead OC, Waniewski RA, Martin DL. Analysis of aspartate and glutamate in human cerebrospinal fluid by high-performance liquid chromatography with automated precolumn derivatization. *Anal Biochem* 1986; 158: 79–86.
31. Plotsky PM, Owens MJ, Nemeroff CB. *Neuropsychopharmacology: The Fourth Generation of Progress*. New York, NY: Raven, 1995.
32. Kaplan JR, Fontenot MB, Berard J, Manuck SB, Mann JJ. Delayed dispersal and elevated monoaminergic activity in free-ranging rhesus monkeys. *Am J Primatol* 1995; 35: 229–234.
33. Coplan J, Karim A, Gupta N, et al. 981. Hypothalamic pituitary-adrenal axis homeostasis in response to chronic foraging uncertainty in vulnerable macaque mothers: contrasting individual versus social allostasis. *Biol Psychiatry* 2017; 81: S397.
34. Kinnally EL, Feinberg C, Kim D, Ferguson K, Coplan JD, Mann J. Transgenerational effects of variable foraging demand stress in female bonnet macaques. *Am J Primatol* 2013; 75: 509–517.
35. Cahill E, Pascoli V, Trifilieff P, et al. D1R/GluN1 complexes in the striatum integrate dopamine and glutamate signalling to control synaptic plasticity and cocaine-induced responses. *Mol Psychiatry* 2014; 19: 1295.