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Early Life Stress Associated With Increased Striatal *N*-Acetyl-Aspartate: Cerebrospinal Fluid Corticotropin-Releasing Factor Concentrations, Hippocampal Volume, Body Mass, and Behavioral Correlates

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Abstract

Introduction: Using proton magnetic resonance spectroscopy imaging, the effects of early life stress on nonhuman primate striatal neuronal integrity were examined as reflected by *N*-acetyl aspartate (NAA) concentrations. NAA measures were interrogated through examining their relationship to previously documented early life stress markers—cerebrospinal fluid corticotropin-releasing factor concentrations, hippocampal volume, body mass, and behavioral timidity. Rodent models of depression exhibit increases in neurotrophic effects in the nucleus accumbens. We hypothesized that rearing under conditions of early life stress (variable foraging demand, VFD) would produce persistent elevations of NAA concentrations (in absolute or ratio form) in ventral striatum/caudate nucleus (VS/CN) with altered correlation to early life stress markers.

Methods: Eleven bonnet macaque males reared under VFD conditions and seven age-matched control subjects underwent proton magnetic resonance spectroscopy imaging during young adulthood. Voxels were placed over VS/CN to capture nucleus accumbens. Cisternal cerebrospinal fluid corticotropin-releasing factor concentrations, hippocampal volume, body mass, and response to a human intruder had been previously determined.

Results: VFD-reared monkeys exhibited significantly increased NAA/creatine concentrations in right VS/CN in comparison to normally reared controls, controlling for multiple comparisons. In comparison to controls, VFD cerebrospinal fluid corticotropin-releasing factor concentrations were directly associated with right VS/CN absolute NAA. Left hippocampal volume was inversely associated with left VS/CN NAA/creatine in VFD reared but not in controls. Disruption of a normative inverse correlation between left VS/CN NAA and body mass was noted in VFD. Only non-VFD subjects exhibited a direct relationship between timidity response to an intruder and right VS/CN NAA.

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Conclusion: Early life stress produced persistent increases in VS/CN NAA, which demonstrated specific patterns of association (or lack thereof) to early life stress markers in comparison to non-VFD subjects. The data are broadly consistent with a stable nonhuman primate phenotype of anxiety and mood disorder vulnerability whereby in vivo indicators of neuronal integrity, although reduced in hippocampus, are increased in striatum. The findings may provide a catalyst for further studies in humans and other species regarding a reciprocal hippocampal/nucleus accumbens relationship in affective disorders.

Keywords
caudate nucleus, nucleus accumbens, striatum, *N*-acetyl-aspartate, hippocampal volume, corticotropin-releasing factor, early life stress

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Introduction

Enduring effects of early life stress (ELS), including reduced neuronal integrity in the anterior cingulate cortex (ACC)[1] and reduced left hippocampal volume,[2] are evident in ELS-exposed nonhuman primates. Nonhuman primates are reared under a schedule of variable maternal ease of access to food,[1,3] termed variable foraging demand (VFD).[4] The VFD paradigm is designed not to affect the quantity of food obtained by mother or the weight trajectory of her developing infant.[4] VFD is experimentally structured to increase maternal foraging unpredictability, which disrupts the integrity of the maternal repertoire.[5] Cerebrospinal fluid (CSF) corticotropin-releasing factor (CRF) concentrations, a stress-related neuropeptide,[6] provide a valid biomarker of VFD rearing. CSF CRF concentrations increase in synchrony in both mother and infant in response to VFD exposure.[7] A persistent elevation of CSF CRF concentrations is then observed in the offspring at the juvenile phase of development and is sustained into full adulthood.[4,8] Moreover, the aforementioned biological features observed in VFD are associated with an anxious/depressed phenotype across the life cycle.[2]

The hippocampus is regarded as the primary site where antidepressants induce neuroprotective effects through, at least in part, increased expression of neurotrophic factors, including brain-derived neurotrophic factor (BDNF).[9,10] Induction of BDNF expression by antidepressants in the hippocampus has demonstrable antidepressant effects,[10] while decreased BDNF expression in humans is correlated with smaller left hippocampal volume in first-episode psychosis.[11] Proton magnetic resonance spectroscopy imaging (^{1}H-MRSI)[1] quantifies alterations in neuronal integrity through measurement of the ubiquitous neurometabolite *N*-acetyl-aspartate (NAA).[12] Spectroscopic imaging (SI) in normal humans confirmed an association between the met-BDNF variant (Rs626), which impairs BDNF tracking and lowers depolarization-induced secretion,[13,14] and reduced hippocampal levels of NAA,[15] suggesting a direct relationship between BDNF function and hippocampal NAA. NAA has also been used as a surrogate marker for clinical evidence of neurotrophic effects of BDNF following intrathecal administration in patients with amyotrophic lateral sclerosis.[16] Moreover, BDNF serum concentrations were predicted by the concentration of NAA in the ACC, indicating that neuronal integrity in the ACC, as reflected by a high concentration of NAA, might be related to high concentrations of BDNF in serum.[17] Consistent with the view that the VFD paradigm models long-standing affective distress[18] and resembles the biology of depressive susceptibility, we previously reported decreased hippocampal NAA concentrations, a marker of neuronal integrity, in VFD-reared subjects in comparison to controls, suggesting decreased hippocampal neurotrophic milieu.[3]

In contrast to the hippocampus, where neurotrophic processes exerts antidepressant-like effects, intraventral tegmental area (VTA) BDNF administration exerts a depression-like effect in the forced swim test (i.e. floating), while blockade of BDNF action in the nucleus accumbens (NAcc) causes an antidepressant-like effect (i.e., struggling).[19] Certain studies report that the increase in neurotrophic effects in the NAcc is critical to the development of a behavioral repertoire associated with social defeat[20] a mouse model of a depression-like phenotype.[19]

The NAcc is a subcortical region of the brain activated during reward/punishment mismatch and is contained within the ventral striatum (VS) of the basal ganglia.[21] In the current study, we utilized ^{1}H-MRSI to determine NAA levels in the macaque VS/caudate nucleus (CN), a region of interest (ROI) intended to encompass the NAcc. To the extent that the VFD model resembles a depression-like analogue,[18] and taken together with other translational studies described above, we hypothesized that NAA concentrations would be *elevated* in VS/CN of macaques who had undergone ELS. Although ^{1}H-MRSI has previously been utilized in human developmental disorders,[22] this is the first report, to our knowledge, that specifically examines VS/CN NAA following

ELS in the nonhuman primate. However, it should be noted that although a body of preclinical and clinical work supports a direct relationship between hippocampal neurotrophic milieu and NAA,[23] no data, to our knowledge, support the converse—that high NAA in the VS/CN ROI is reflective of an increase in regional neurotrophic milieu. Interestingly, we have reported that patients with major depressive disorder (MDD) exhibit hypertrophy of the NAcc.[24] Moreover, antidepressant response to ketamine predicted reduction in NAcc volume and an increase in hippocampal volume at 24-h postadministration.[24]

We posed a second hypothesis—whether timid behavioral responses to a human intruder would directly relate to VS/CN NAA, particularly in a VFD-reared group. Timid vis-à-vis confrontational responses to the human intruder provides a behavioral profile which aligns with markers of ELS,[2] and timidity responses are argued to provide a nonhuman primate proxy of anxiety-like behaviors.[2]

Based on reports demonstrating reciprocal roles for BDNF in the hippocampus, where it mediates antidepressant-like effects,[10] and VTA-NAcc, where social defeat enhances BDNF expression,[25] we hypothesized that the putative increases in VS/CN NAA would be associated with reductions in hippocampal volume, specifically in VFD-reared subjects. Of note, we previously reported reduced left hippocampal volume in VFD-reared animals in comparison to unstressed controls.[2,3] Therefore, an inverse correlation between VS/CN NAA and hippocampal volume specifically in subjects exposed to ELS was hypothesized to support the reciprocal nature of neurotrophic influences on the VS/CN versus hippocampus.

CRF exerts pronounced effects on striatal-dependent behaviors such as facilitating drug addiction, disruption of pair-bonding, and interruption of natural reward, each through modulation of the NAcc.[26] CRF immunostaining is evident in dense concentrations in the NAcc.[27] However, neither acute nor chronic stress increases CRF-like immunoreactivity within the NAcc.[28] It is clear, nevertheless, that CRF interacts with dopamine neurotransmission under conditions of stress to enhance drug craving.[29] Increased central CRF expression has been documented following VFD rearing[30] and is implicated in mood disorders.[31] A positive association between VS/CN NAA and CRF activation was hypothesized.[25]

Increased neurotrophic activity in the VTA-NAcc pathway may also cause profound weight loss,[32] an effect attributed to a hyperdopaminergic (e.g., amphetamine-like) state. The latter may cause reductions in the rewarding properties of food.[33] Developmental stress has been postulated to exacerbate markers of metabolic syndrome,[34] and our previous studies have shown that VFD rearing resulted in increased body weight, body mass index (BMI), and abdominal circumference.[35] Moreover, CSF CRF concentrations in VFD-reared juveniles directly predicted adult BMI.[36] Therefore, in addition to causing an anxious/depressed phenotype, the VFD form of ELS causes an increase in both central CRF and body mass. Thus, we hypothesized an inverse association between VS/CN NAA with body mass.

In sum, consistent with a body of data regarding VTA/NAcc in negative affective states, we hypothesized that NAA concentrations in voxels placed over VS/CN would be increased following ELS in the form of VFD rearing. The association of VS/CN NAA concentrations to CSF CRF (positive), hippocampal volumes (inverse), metabolic indices (inverse), and timid behavioral responses to a human intruder (direct) warranted examination in the VFD model versus controls. Positive preclinical studies of VS/CN NAA increases following ELS may translate to clinical MRSI studies.

Methods

Eighteen bonnet macaque (*Macaca radiata*) males were selected for this study (4 of 22 subjects scanned did not have MRSI data available due to technical difficulties). Eleven reared under VFD conditions were age-matched to seven control subjects and underwent ^{1}H-MRSI, magnetic resonance imaging (MRI) for volumetrics,[2] cisternal CSF taps for CRF concentrations,[30] and morphometric examination.[35] Rearing groups were not distinguishable by age (VFD mean (standard deviation, *SD*) = 60.56 (33.82) months vs. non-VFD mean (*SD*) = 51.27 (7.69); t value = −0.71; df = 16; p = .49) or weight (VFD mean (*SD*) = 4.93 (1.35) kg vs. non-VFD mean (SD) = 4.29 (0.59); t value = −1.71; df = 16; p = .26). The subjects had participated in previous reports,[2,30,35] but ^{1}H-MRSI studies of NAA in the VS/CN region have not previously been reported.

VFD Rearing

Maternal-infant dyads were randomly assigned, the latter to control for treatment conditions, shortly after birth. Beginning when their infants were at least 12 weeks old, mothers confronted 16 weeks of foraging conditions in which the time and effort required to obtain food were either relatively brief and easy (low foraging demand (LFD); essentially ad libitum access) or more lengthy and difficult (high foraging demand (HFD)). This alternating of periods of LFD and HFD in two-week blocks is termed VFD. All subjects have ample food throughout the study, confirmed by frequent weight and health checks, which ensured normal growth and development in both mothers and infants.[4] After the experimental period and at the time of testing, all offspring are on ad libitum feeding.

CSF Sampling. Previously reported data on CSF CRF concentrations obtained when the offspring were adolescents at a mean of 2.7 years old[30] were available. The time

between the end of the VFD procedure and the CSF sampling was about two to three years. In brief, subjects were taken from their home cages and placed in carrying cages, a routine procedure. For CSF sampling, subjects were released into restraint cages and intramuscular ketamine (15 mg/kg) was administered. Cisternal CSF samples were obtained and then placed in Gant tubes and stored in a −70°C freezer.[4] Assays for CRF were performed according to the methods described in Nemeroff et al.[37] The assay has a sensitivity of 2.5 pg per tube and intra- and interassay coefficients of variation of 3%–6% and 10%–13%, respectively. The laboratory personnel conducting the CRF radioimmunoassays were blind to the rearing status of the subjects.

Scanning Procedures

Animal Procedures. As described previously, on the day of the brain scan study, subjects were ushered into familiar carrying cages and transported to Mount Sinai Medical Center in a dedicated animal transport van with air-conditioning.[2] Upon arrival at the scanner, animals were moved to a squeeze cage and following a brief restraint period, rapidly given anesthetic agent intramuscularly. Saffan®, previously known as CT1341, is an injectable veterinary steroid anesthetic and minimizes motion artifact, relative to ketamine. Saffan® was administered at a dose of 16 mg/kg, which comprises two bioactive constituents: 12 mg/kg of alphaxalone and 4 mg/kg alphadolone acetate. Once sedated, the monkeys' heads were positioned in a Styrofoam headrest inside a human knee coil and taped snugly over the forehead to minimize movement. Subjects remained anesthetized throughout scanning and were continuously monitored by pulse oximeter. Infrequently, because of evidence of motion during the scan due to diminished level of anesthesia, animals necessitated subsequent doses of Saffan® (¼ initial dose). Subjects usually awakened within 20 min following completion of the 1-h scan. Following the imaging procedures, subjects returned on the same day to their home cages.

Magnetic Resonance Imaging. MRI data were acquired in a 3-T Siemens Allegra scanner. The protocol for the structural scans consisted of a three-plane sagittal localizer from which all other structural scans were prescribed. The following structural scans were acquired: axial 3D-MPRAGE (TR = 2500 ms, TE = 4.4 ms, field of view (FOV) = 21 cm, matrix size = 256 × 256 yielding 208 slices with thickness = 0.82 mm); Turbo spin echo T2-weighted axial (TR = 5380 ms, TE = 99 ms, FOV = 18.3 cm × 21 cm, matrix size = 512 × 448, turbo factor = 11, 28 slices, thickness = 3 mm, skip 1 mm).

Proton Magnetic Resonance Spectroscopy Imaging. Localizer magnetic resonance images for prescribing the MRS volumes consisted of a T1 sagittal with the following parameters: TR = 500 ms, TE = 10 ms, FOV = 18 cm × 14 cm, matrix size = 512 × 384, 4.3 mm thick with 1.1 mm spacing. Twenty-five slices were obtained to cover the whole brain. From the sagittal image, one T1-weighted transverse slice (TR = 500 ms, TE = 10 ms, thickness = 10 mm, FOV = 16.5 cm × 22 cm with matrix size 512 × 384) was identified for MRS acquisition. A nearly axial plane was chosen for the plane going through the CN. It is chosen to be parallel to the AC–PC line. ^{1}H SI data of the left and right caudate nuclei intended to include VS (see voxel placement in Figure 1) were obtained in two sequential scans using the phase-encoded version of the standard PRESS volume localization sequence, with TR = 2000 ms, TE = 30 ms, 24 × 24 phase-encoding steps over an FOV of 16 cm (zero filled to 32 × 32 phase-encoding steps before 3D Fourier transformation), a slice thickness of 10 cm slice, 1 average per phase-encoding step and circular k-space sampling, to obtain voxels having a nominal size of 0.25 cm^3 (1.0 × 0.5 × 0.5 cm^3). Outer volume saturation bands were prescribed to coincide with all eight sides of the PRESS box. Water suppression and magnet shimming were automatically performed and adjusted by the host computer. The raw SI data were processed and fitted in the frequency domain to obtain metabolite peak areas using manufacturer-supplied MRS data processing software. Individual CSI images were reconstructed and overlaid onto the T1 anatomical images. Automatic phase correction was applied, voxels of interests were

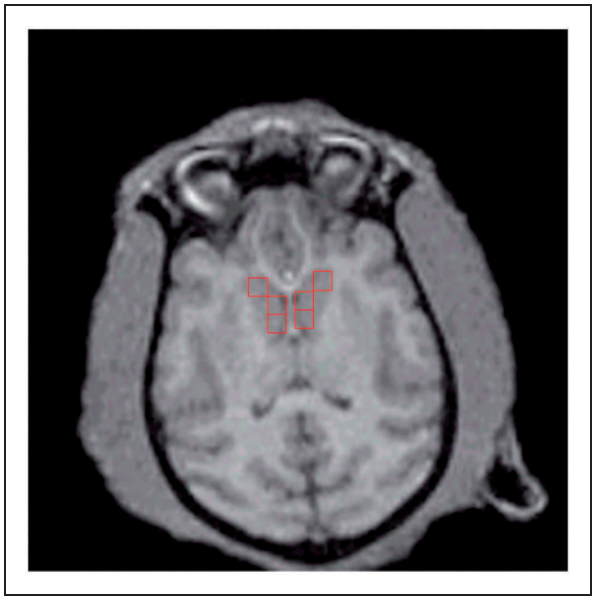

Figure 1. Voxel placement on an MRI-acquired coaxial plane for acquisition of spectral signals from ventral striatum/caudate nucleus. Examples of spectral signals acquired in nonhuman primates using similar ^{1}H-MRSI methodology are available in Matthew et al. (2).

identified, and the metabolite levels were derived from the spectral fits. ^{1}H-MRSI metabolites (NAA, choline (Cho), Cr) were obtained from the ROIs (see Mathew et al.[1]). Voxels with poor spectral data quality, defined as unresolved Cr and Cho resonances, were excluded from analysis. The metabolites from the selected voxels in each of the ROIs were averaged and transferred to Statistica v6 (StatSoft Inc., Tulsa, OK) for statistical analysis.

MRI Data Preprocessing and Analysis for Hippocampal Volume. All MRI ROI data analyses were completed by raters blind to subjects' rearing. The axial MPRAGE series were imported into ANALYZE AVW7.0 software platform. In order to isolate whole brain from its surroundings, skull, surface CSF, and meninges were stripped using a combination of tools including image thresholding, region growing, and manual tracing.

The hippocampi were manually traced bilaterally using a detailed set of guidelines developed by Schumann et al.[38] and adjusted, when necessary, to the bonnet macaque brain morphology using a primate brain atlas. The tracings were performed in oblique coronal slices but were also checked in sagittal and axial views. Repeated measurements were performed in a random order on five subjects, and both intrarater and interrater reliability gave an ICC of 0.93 for right/left hippocampus. An additional structural MRI data analysis was performed using VBM SPM5 (Wellcome Department of Imaging Neuroscience, London, UK; www.fil.ion.ucl.ac.uk/spm). Briefly, a single subject raised under normative conditions was chosen as the initial template. All images were registered through linear (zooms, rotations, translations, and shears) deformations to the single-subject template. An average image, called template henceforth, was created with the obtained deformed images. Afterward, the same original images were linearly deformed to the created template, and this step was iterated 20 times to minimize the bias caused by utilization of a single subject as the initial template. On the 22nd step, original images were linearly and also nonlinearly registered to the final template. A brain mask containing gray matter, white matter, and CSF was manually delineated for the template and used to eliminate skull and meninges from the final registered images. In order to preserve brain volume, images were scaled using the Jacobian matrix, so that the total amount of gray matter in the resulting images remains the same as it would be in the original images. The obtained images were finally smoothed with a Gaussian filter at full width at height maximum equal to 4 mm.[2]

Behavioral Response to a Human Intruder. Sixteen of 18 animals that participated in the ^{1}H-MRSI study were subject to behavioral testing during late adulthood, approximately three years after neuroimaging. The animals were exposed briefly to a human intruder, a fear stimulus that is a variation of a previously detailed masked intruder paradigm.[18] Emotional reactivity was rated by two experimenters blind to rearing status using a three-point scoring scale. To receive a score of "1" for intruder distress, subjects exhibited "confrontational" behaviors including fang-baring, growling, direct eye contact, piloerection, ear flexing, cage shaking, and mouth gaping. A "timid" response received a score of "3," which was characterized by an animal that was minimally confrontational, averting eye contact, submissive and displaying lip-smacking, and receding to the back of the cage. A score of "2" describes a subject with intermediate or alternating levels of both confrontational and timid behaviors. One hundred percent interrater reliability was observed for the intruder behavioral scoring system.

Statistical Methods

Data were inspected for outliers and tested for normality of distribution for left and right VS/CN absolute NAA and left and right VS/CN NAA/Cr (creatine), which were the primary independent measures of study. Predictor variables (CSF CRF, hippocampal volume, body mass, and timidity response to an intruder) were also evaluated for normality. Rearing group effects were analyzed by t test between VFD and non-VFD monkeys. We examined whether age and weight were distinguishable either by rearing groups, or whether either variable was predictive of the dependent or predictor variables in the current study. In either of these instances, age and/or weight were used as covariates. General linear models (GLMs) were used where the predictor variable was the variable of interest (CSF CRF concentrations, right or left hippocampal volume, body mass, and behavioral response to an intruder) and left and right VS/CN absolute NAA and left and right NAA/Cr served as a 2×2 (NAA measure (absolute vs. ratio) $\times$ VS/CN side) repeated measures dependent variable. The interaction term between rearing group and the predictor variable was included in the GLM and, when significant, would indicate an associative relationship between the predictor and predicted variable that was significantly influenced by rearing status. Univariate analyses followed and the interactive term of individual NAA measures was the primary focus (Appendix Tables 4–7). Within-rearing group, Pearson's correlational matrices were computed using left and right absolute NAA and left and right NAA/Cr, correlated with the measures of interest for ELS.

The GLM makes an assumption of a linear response model and normal distribution, so a generalized linear model with 95% confidence limits (CL) was employed to confirm the interactive results. The latter test allows for response variables that have error distribution models other than normal distribution (arbitrary distributions) to be analyzed.[39] All tests were two-tailed. Since the primary

hypothesis was that VS/CN NAA or NAA/Cr concentrations were elevated in VFD subjects versus non-VFD controls, and four NAA measures were available (left and right, and absolute NAA and NAA/Cr), data were corrected for four comparisons; $p \leq .0125$. Because of the exploratory nature of the associative relations, significance for these tests was set at a significance level of $p \leq .05$, two-tailed. To protect against type I errors, an effect size of the interactive term would need to reach twice that of a large effect size as determined by partial η^2.[40]

The Akaike information criterion (AIC)[41] is an estimator of the relative quality of statistical models for a given set of data. Given a variety of models for a data set, AIC provides an estimate of the quality of each model, relative to each of the other models. Burnham and Anderson[42] note that, since AIC corrected (AICc) converges to AIC as N gets large, AICc—rather than AIC—should generally be employed. To provide a context for the "goodness of fit" of the AICc, we performed generalized linear models for bivariate correlations in order to provide a context for the AICc values and to confirm the key Pearson's correlations, which assume normality of distribution of the response variable. In addition, Appendix Table 8 provides the AIC, AICc, and Bayesian information criterion (a variation on AIC) for each of the four generalized models conducted (see Appendix Table 8).

Results

All dependent and predictor variables were normally distributed except for CSF CRF (Kolmogorov–Smirnoff $d = .27$, $p < .10$; Lilliefors $p < .01$), and no outliers were noted. CSF CRF values were therefore logarithmically transformed. Body mass did not correlate significantly with each of the four NAA variables (p all $> .44$). Body mass, however, was observed to predict intruder status at a trend level ($r = -.38$; $p = .087$), but no correlation was observed for log CSF CRF or left or right hippocampal volume (all $p > .44$). Body mass was therefore used as a covariate for the intruder response analysis. No age effects were noted.

VS/CN NAA Rearing Group Comparisons

VFD rearing was associated with a significant increase in right VS/CN nucleus NAA/Cr in VFD subjects when compared to non-VFD monkeys (VFD mean $(SD) = 1.76$ (0.23) vs. non-VFD mean $(SD) = 1.50$ (0.12); t value $= -2.78$; $df = 16$; $p = .01$; see Table 1). The result remained significant following correction for multiple comparisons. The remaining three comparisons (left and right absolute NAA and left NAA/Cr) were all nonsignificant ($p \geq .20$). Of note, one VFD subject's values for left VS/CN were unavailable due to technical difficulties.

Table 1. Means and standard deviations of NAA measures obtained from ^{1}H-MRSI of the ventral striatum/caudate nucleus with student t tests.

VS/CN	Non-VFD, mean ± *SD* (N = 7)	VFD, mean ± *SD* (N = 11)	t value	df	p
L NAA	1.49 ± 0.46[a]	1.58 ± 0.33	−0.47	15	0.64
L NAA/Cr	1.55 ± 0.33[a]	1.59 ± 0.29	−0.26	15	0.80
R NAA	1.56 ± 0.44	1.84 ± 0.45	−1.33	16	0.20
R NAA/Cr	**1.50 ± 0.12**	**1.76 ± 0.23**	**−2.78**	**16**	**0.01**

Note: Significant results are shown in bold. VS/CN: ventral striatum/caudate nucleus; VFD: variable foraging demand; NAA: *N*-acetyl-aspartate; Cr: creatine; *SD*: standard deviation.
[a]Data on one subject missing. See text for full analyses.

GLM for VS/CN NAA Associations

CSF CRF Concentrations. An overall group × log CSF CRF × hemispheric interaction was observed ($F_{(1,12)} = 8.41$; $p = .013$). Univariate analyses revealed a group × log CSF CRF interaction for right absolute NAA ($F_{(1,12)} = 7.35$; $p = .019$; partial $\eta^2 = 0.45$; see Appendix Table 4). Log CSF CRF concentrations exhibited a direct correlation with right absolute NAA in VFD-reared macaques ($r = .71$; $N = 9$; $p = .03$) by contrast the corresponding correlation in non-VFD was directionally inverse (see Table 2). GLM univariate results were confirmed by generalized linear models (Wald statistic $(df = 1) = 6.37$, $p = .01$, lower CL 95.0% $= -0.81$; upper CL 95.0% $= -0.10$). Generalized linear models revealed a markedly significant relationship between Log CRF and right VS/CN absolute NAA in VFD subjects (Wald statistic $(df = 1) = 11.93$, $p = .0005$). AICc was 13.72 which ranked third of four key correlations (see Table 3).

Hippocampal Volume. An overall NAA measure × group × left hippocampal volume interactive effect was observed at a trend level ($F_{(1,12)} = 3.99$; $p = .068$). Univariate testing revealed a group × left hippocampal volume interaction ($F_{(1,12)} = 9.90$; $p = .008$; partial $\eta^2 = 0.45$) whereby the relationship between left VS/CN NAA/Cr ratio and left hippocampus differed as a function of rearing. In VFD-reared subjects, there was an inverse relationship between left VS/CN NAA/Cr and left hippocampal volume ($r = -.79$; $N = 9$; $p = .011$), whereas a directionally positive relationship was noted in non-VFD (see Table 2). Moreover, in VFD, left VS/CN absolute NAA was inversely correlated with left hippocampal volume ($r = -.82$; $N = 9$, $p = .007$; see Table 2), but the overall GLM was not significant (see Appendix Table 5). No effects were evident for right hippocampus. GLM univariate results

Table 2. Comparison of Pearson's correlation categorized by rearing group and examining ELS markers versus left and right VS/CN NAA and NAA/Cr.

	VFD				Non-VFD			
	Left (N = 9)		Right (N = 9)		Left (N = 7)		Right (N = 7)	
	ABS NAA	NAA/CR	ABS NAA	NAA/CR	ABS NAA	NAA/CR	ABS NAA	NAA/CR
CSF CRF(ng/ml) log	**.71**	.13	**.81**[a]	.22	−.35	.67	−.59[a]	−.47
	p = .03	p = .73	**p = .008**	p = .56	p = .42	p = .095	p = .15	p = .28
Left hippocampus (cm^3)	**−.82**	**−.79**[b]	−.29	−.52	−.070	.63[b]	−.43	−.18
	p = .007	**p = .011**	p = .46	p = .15	p = .88	p = .12	p = .33	p = .68
Right hippocampus (cm^3)	−.51	−.20	−.09	−.15	−.15	.70	−.51	−.36
	p = .15	p = .60	p = .81	p = .70	p = .74	p = .07	p = .23	p = .42
Body mass (kg)	.72[c]	.36	−.22	−.23	**−.85**[c]	−.24	**−.79**	**−.86**
	p = .028	p = .33	p = .58	p = .55	**p = .013**	p = .59	**p = .032**	**p = .012**
Timidity response × to intruder	−.01	.00	.14[d]	−.23	.74[e]	−.26	**.90**[d]	.6
	p = .98	p = .99	p = .72	p = .56	p = .09	p = .61	**p = .016**	p = .20

Note: Significant results are bolded. Of note, one VFD subject's values for left VS/CN nucleus were not unavailable due to technical difficulties. VFD: variable foraging demand; ABS NAA: absolute *N*-acetyl-aspartate; NAA/Cr: ratio of *N*-acetyl-aspartate/creatine; CSF CRF: cerebrospinal fluid corticotropin releasing-factor concentrations; Conc.: concentrations.

[a]Group × log CSF CRF conc. interaction ($F_{(1,12)} = 7.35$; $p = .019$; partial $\eta^2 = 0.45$).

[b]Group × left hippocampal volume interaction ($F_{(1,12)} = 9.90$; $p = .008$; partial $\eta^2 = 0.45$).

[c]Group × body mass interaction ($F_{(1,13)} = 12.24$; $p = .004$; partial $\eta^2 = 0.48$).

[d]Group × behavioral response interaction ($F_{(1,10)} = 10.57$; $p = .009$; partial $\eta^2 = 0.51$).

[e]N = 6 for non-VFD behavioral response to an intruder.

Table 3. Aikake information criterion (AIC), AIC corrected for sample size, Bayesian information criterion, and generalized linear models of bivariate analyses.

	Distribution: NORMAL link function: LOG			
Statistic	Log CRF/right ABS NAA VFD	Left hipp./left NAA/Cr VFD	Weight/left ABS NAA non-VFD	Timidity/right ABS NAA non-VFD
AIC	9.72	−0.35	2.85	5.33
AICc	13.72	4.44	10.85	17.33
BIC	10.62	0.23	2.69	4.70
Wald statistic (df = 1)	**11.93; p = .0005**	**13.03; p = .0003**	**30.27; p = .00001**	**13.12; p = .0003**

Note: The AICc is employed specifically to evaluate the "goodness of fit" for a given data set and a means of model selection for minimizing loss of data. In the current paper, the data sets for each of the generalized linear regression analyses are different. Nevertheless, the best model is judged as the model with the smallest AIC. The table indicates that the generalized linear models for bivariate analyses are highly significant. The AICc shows the "best" model is for left hippocampus/left NAA/Cr, followed by weight/left ABS NAA, Log CRF/right ABS NAA and lastly by timidity/ Right ABS NAA. VFD: variable foraging demand; ABS NAA: absolute *N*-acetyl-aspartate; NAA/Cr: ratio of *N*-acetyl-aspartate/creatine; CRF: corticotropin releasing-factor; AIC: Akaike information criterion; BIC: Bayesian information criterion; AICc: AIC corrected for finite sample sizes; hipp. = hippocampus.

were confirmed by generalized linear models (Wald statistic (df = 1) = 13.53, p = .0002, lower CL 95.0% = 2.53; upper CL 95.0% = 8.29). Generalized linear models revealed a markedly significant relationship between left hippocampus and left VS/CN NAA/Cr in VFD subjects (Wald statistic (df = 1) = 13.03, p = .0003). AICc was 4.44 which ranked first of four key correlations (see Table 3).

Body Mass. An overall rearing group × body mass effect was observed ($F_{(1,13)} = 7.37$; $p = .017$); There was a rearing group × body mass interaction for left VS/CN absolute NAA ($F_{(1,13)} = 12.24$; $p = .004$; partial $\eta^2 = 0.48$; Appendix Table 6; Figure 3) reflecting a significant inverse relationship in controls between body mass and left VS/CN absolute NAA, but a direct relationship in VFD ((r = .72; N = 9; p = .028 (see Figure 2)). Thus, VFD rearing altered the normative inverse relationship between left VS/CN absolute NAA and body mass. For body mass, there were a number of inverse relationships in the non-VFD subjects (left VS/CN

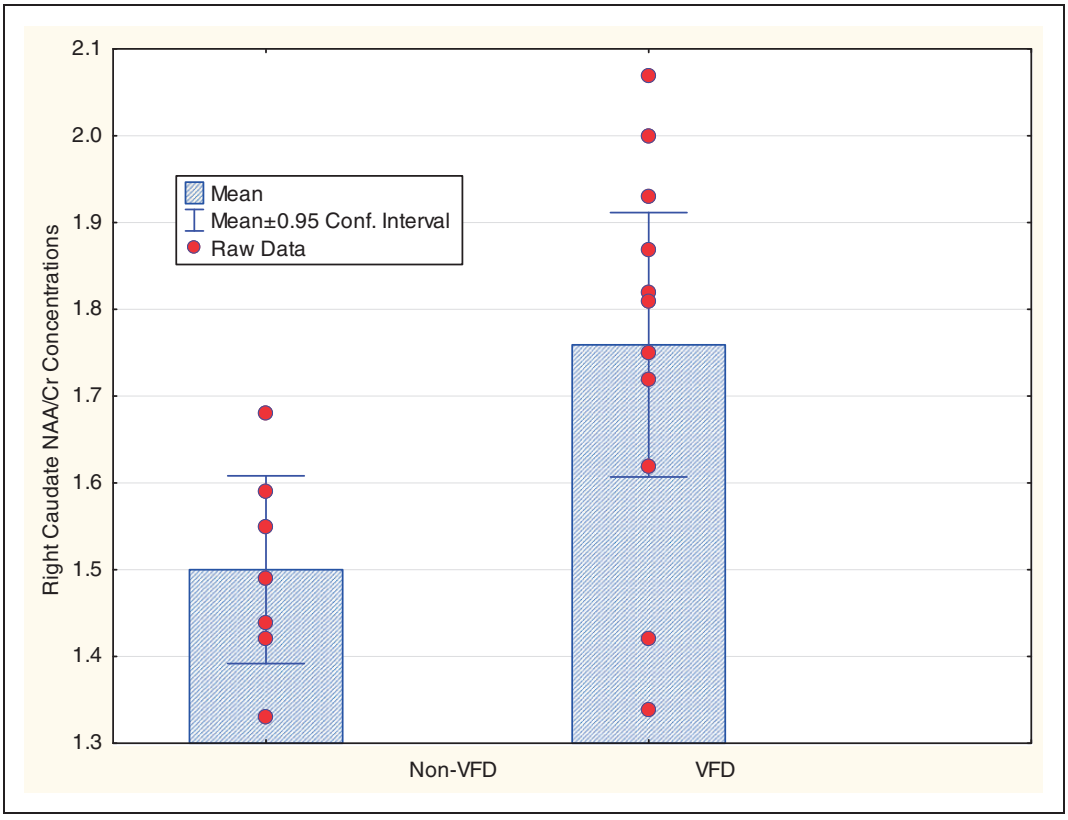

Figure 2. Effect of early life stress on right ventral striatum/caudate nucleus *N*-acetyl-aspartate/creatine. VFD rearing was associated with a significant increase in the NAA/Cr concentrations in the right VS/CN when compared to non-VFD monkeys (VFD mean (*SD*) = 1.76 (0.23) vs. non-VFD mean (*SD*) = 1.50 (0.12); t value = −2.78; df = 16; p = .01). The result remained significant following correction for multiple comparisons ($p \leq .0125$). The remaining three comparisons (left and right absolute NAA and left NAA/Cr) were all nonsignificant ($p \geq .20$). NAA/CR: ratio of *N*-acetyl-aspartate/creatine; ^{1}H-MRSI: proton magnetic resonance spectroscopic imaging; VFD: variable foraging demand.

absolute NAA: r = −86; N = 7; p = .013; right VS/CN absolute NAA (r = −79; N = 7; p = .032) and right VS/CN NAA/Cr (r = −.87; N = 7; p = .012; Table 2).

The group × weight interactive effect was confirmed by generalized linear models (Wald statistic (df = 1) = 20.51, p = .000006, lower CL 95.0% = −0.37; upper CL 95.0% = −0.15). Generalized linear models revealed a markedly significant relationship between body mass and left VS/CN Absolute NAA in non-VFD subjects (Wald statistic (df = 1) = 30.27, p = .00001). AICc was 10.85 which ranked second of four key correlations (see Table 3).

Response to Intruder Stress

Body mass clearly had an influence on VS/CN NAA as a function of rearing (see above), and therefore, body mass was used as a covariate in the intruder response analysis. Moreover, as described above, there was a significant group × body mass interaction in the prediction of VS/CN NAA measures. We therefore developed a GLM model controlling for body mass as a covariate, used behavioral response to a human intruder as a continuous predictor variable, and controlled for the body mass × group interactive effect by introducing a triple interaction variable—intruder response × body mass × group. Controlling for the body mass effect ($F_{(1,10)} = 6.23$; p = .032; greater body mass = less timid), there was a group × behavioral response interactive effect ($F_{(1,10)} = 10.57$; p = .009; partial $\eta^2 = 0.51$). The latter effect was significant when controlling for a triple interactive term which included body mass (group × body mass × behavioral response effect ($F_{(1,10)} = 8.28$; p = .016)). Right VS/CN absolute NAA was directly associated with timidity responses to an intruder in non-VFD (r = .90; N = 6; p = .016), but the corresponding correlation was not significant in VFD ($F_{(1,10)} = 11.37$; p = .007; Figure 4; Table 2; see Appendix Table 7).

The group × timidity interactive effect for right VS/CN absolute NAA was confirmed by generalized linear models (Wald statistic (df = 1) = 5.19, p = .02, lower CL 95.0% = 0.03; upper CL 95.0% = 0.35). Generalized

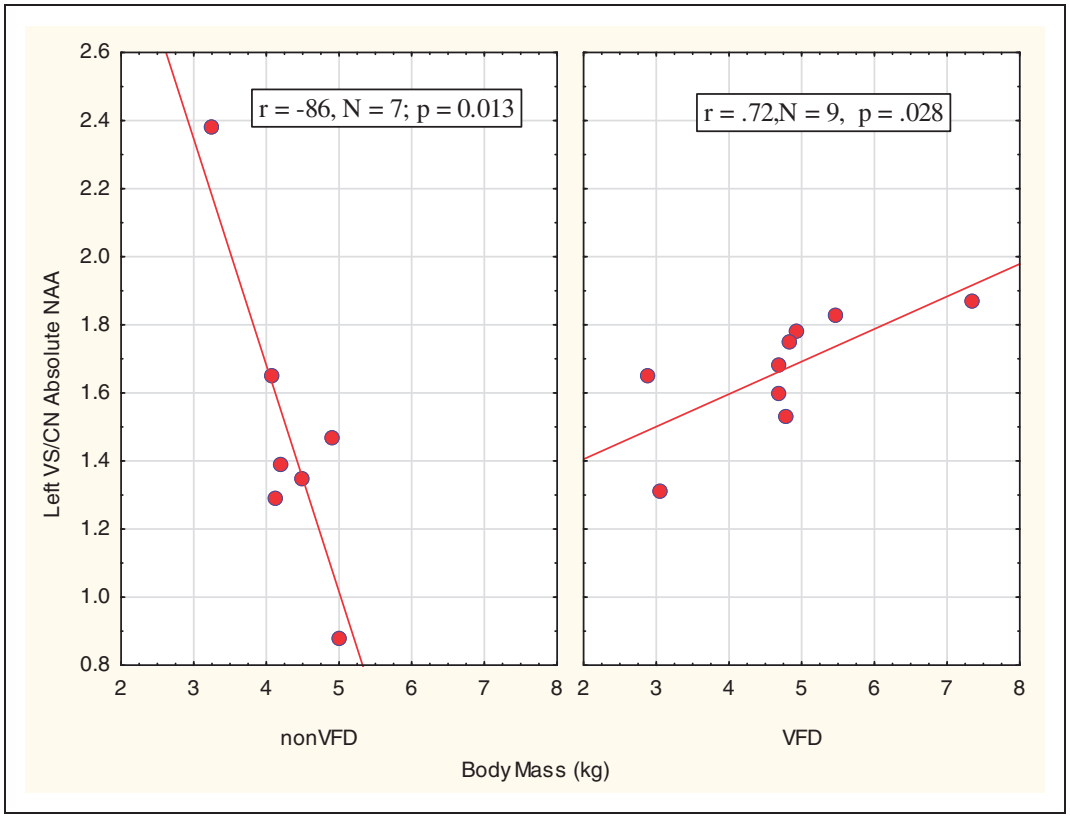

Figure 3. Early life stress alters the relationship between body mass and left ventral striatum/caudate nucleus absolute *N*-acetyl-aspartate concentrations. There was a rearing group × weight interaction for left VS/CN absolute NAA ($F_{(1,13)} = 12.24$; $p = .004$; partial $\eta^2 = 0.48$) reflecting a significant inverse relationship between body mass and left VS/CN absolute NAA ($r = -86$; $N = 7$; $p = .013$) but a direct relationship in VFD ($r = .72$; $N = 9$; $p = .028$). Thus, VFD rearing produced an altered relationship between the normative inverse relationship between left VS/CN absolute NAA and body weight. VS/CN: ventral striatum/caudate nucleus; VFD: variable foraging demand; NAA: *N*-acetyl aspartate.

linear models revealed a markedly significant relationship between timidity and right VS/CN Absolute NAA in non-VFD subjects (Wald statistic ($df = 1$) = 13.12, $p = .0003$). AICc was 17.33 which ranked fourth of four key correlations (see Table 3).

Discussion

The data of the current study indicate that VFD-reared bonnet macaques exhibit significantly increased right VS/CN NAA/Cr in comparison to normally reared controls, an effect that retained significance following correction for multiple comparisons. Based on the finding of an increased neurotrophic response in mouse NAcc following social defeat stress,[20] NAA measures, shown to be representative of neuronal integrity,[16,18] would be expected to be relatively elevated in striatum following ELS. To the extent, NAA is deemed to reflect neuronal integrity, increased NAA in the VS/CN may conceivably reflect a persistent increase in neurotrophic milieu.[23,43] However, the current study provides no evidence that the increased NAA in VS/CN is related to an increase in regional neurotrophic response, for example, BDNF measures. For instance, an increase in NAA may also reflect increased regional energy metabolism in neuronal mitochondria.[44] However, NAA measures in VS/CN, whether as absolute concentration or as NAA/Cr, were predicted (or not) by relationships between VFD subjects and stress markers, generally occurring in the expected direction.

A group × log CSF CRF concentration interactive effect indicated that CRF concentrations positively predicted right VS/CN absolute NAA, specifically within the VFD group. The correlation was greater than the corresponding correlation in non-VFD subjects (the interactive effect size was in excess of threefold greater than a large effect size (0.14)[40]). Based on prior studies indicating elevations of CSF CRF concentrations in VFD versus non-VFD subjects,[4,30] the data suggested that this effect may occur in concert with VS/CN absolute NAA elevations.

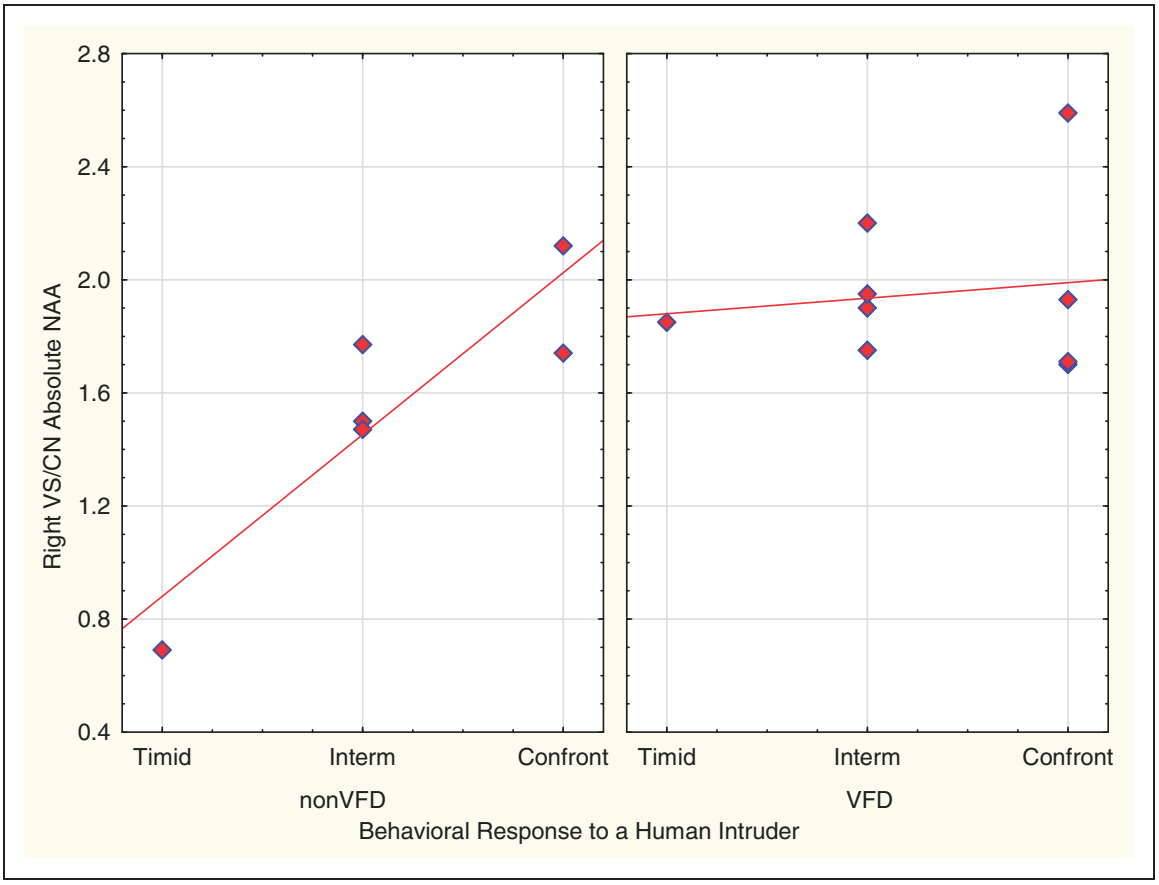

Figure 4. Relationship of timidity responses to a human intruder and right VS/CN absolute NAA. Controlling for body mass effect ($F_{(1,10)} = 6.23$; $p = .032$), there was a group × behavioral response interactive effect ($F_{(1,10)} = 10.57$; $p = .009$; partial $\eta^2 = 0.51$). The latter effect was significant when controlling for a triple interactive (group × body mass × behavioral response effect ($F_{(1,10)} = 8.28$; $p = .016$) to include body mass in the model. Right VS/CN absolute NAA was directly associated with timidity responses to an intruder in non-VFD ($r = .90$; $N = 6$; $p = .016$), but the corresponding correlation was not significant in VFD (see Table 2). VS/CN: ventral striatum/caudate nucleus; VFD: variable foraging demand; NAA: *N*-acetyl aspartate.

Consistent with a reciprocal relationship between NAcc (increased) versus hippocampal (decreased) for neurotrophic expression following social adversity in rodents,[25] left hippocampal volume decreases predicted increases in left VS/CN NAA/Cr, specifically in VFD subjects. Of note, left hippocampal volume in the current cohort was previously demonstrated to be persistently reduced in VFD versus non-VFD subjects.[2] That relatively high VS/CN NAA was associated with reduced left hippocampal volume, specifically in VFD-reared nonhuman primates, potentially provides support for a relationship of reciprocal neuronal entailing VS/CN and hippocampus.

VFD rearing significantly disrupted a "normal" inverse correlation observed in non-VFD animals between body weight and VS/CN NAA concentrations bilaterally. The inverse association between body mass and NAA in non-VFD was significantly distinguishable from an absence of the relationship observed in VFD subjects (see Appendix Table 7, Figure 3). The latter suggested a loss of association between VS/CN NAA and body mass in VFD. An increase in neurotrophic effects in the VTA-NAcc pathway in rats causes profound weight loss,[32] which has been postulated to stem from a hyperdopaminergic (e.g., amphetamine-like) state which causes reductions in the rewarding properties of food.[45] The loss of the relationship, or even frank reversal (see Figure 3), may set the stage for the documented increase in BMI in young adult VFD versus non-VFD subjects.[35] VFD rearing may conceivably interfere with dopaminergic functioning in the NAcc and an uncoupling in VFD of the NAA spectral signal, and body mass may underlie, in part, a positive association between early life adversity and both adult obesity and/or substance abuse.[46] Supportive of the latter "uncoupling" view, when adjusting for body mass, left VS/CN absolute

NAA was elevated in VFD versus non-VFD subjects, suggesting that elevations of NAA were not associated with linear reductions in body mass in VFD-reared subjects. Of note, we have demonstrated in humans that overweight individuals exhibit reduced hippocampal absolute NAA,[47] but we did not examine concomitant NAA concentrations in striatum.

Finally, right VS/CN absolute NAA in non-VFD was positively associated with timidity (Figure 4). The expected relationship between high VS/CN NAA and timidity evident in non-VFD subjects was absent in VFD subjects, again suggesting that VFD rearing may interfere with a relationship linking an adaptive fear-related behavioral response and VS/CN NAA.

All interactive effects for the four predictor variables (log CRF, left hippocampal volume, body mass, and timidity response to a human intruder) were significant when using a generalized linear model, which controls for the possibility of nonnormal distributions (Table 3, Appendix Table 8). Moreover, significant Pearson's correlations were robustly confirmed using the generalized linear model, and AICc values indicated a "closeness of fit" for each of the four key correlations.

The current study therefore links ELS to persistent elevations of VS/CN NAA and demonstrates a positive association between VS/CN NAA and CSF CRF concentrations, specifically in subjects exposed to early life adversity. An inverse association between VS/CN NAA with left hippocampal volume in VFD, but not non-VFD, is consistent with the reciprocal hippocampal/NAcc relationship observed in rodent social defeat studies[2,4] and possibly human volumetric studies of MDD.[24] Additional findings demonstrate a loss in VFD of a normative inverse relationship between VS/CN NAA and body mass. Altered patterns of neuroplasticity in the VS that disrupt endogenous amphetamine-like effects may be implicated in obesity and substance use disorders following early life adversity.[48] VFD-reared subjects also exhibit loss of a normative direct correlation between timidity in response to an intruder and VS/CN NAA, again suggesting a failure to elicit graded behavioral responses following enhanced neuronal integrity in the NAcc.

Limitations of the study include the exploratory nature of the analysis, yet the primary finding of persistent elevations of striatal NAA concentrations in VFD-reared subjects survived correction for multiple testing. In addition, when using body mass as a covariate in a more elaborate GLM, it emerges that there are overall increases in absolute NAA in the VFD versus controls using our ROI of left VS/CN. Moreover, effect sizes yielded by the interaction analyses are observed to occur in the direction expected and are generally threefold greater than the level necessary to generate a large effect size.[40]

The current male macaque sample has been used in previous reports.[2,30] The findings require replication in other male bonnet macaque samples as well as rhesus macaque and female samples generally. However, positive findings arising from multiple stress-related biological systems in the same sample may well indicate an array of coordinated parallel alterations occurring in tandem. We are, however, not aware of any study that provides a precedent for ^{1}H-MRSI studies of the VS following ELS.

Frye et al.[49] reported decreased NAA and NAA/Cr in a voxel containing "basal ganglia" in bipolar disorder patients. Of note, the number of hospitalizations for manic episodes correlated inversely with NAA. The latter finding emphasizes that the VFD model provides insights into the effects of ELS without overlying confounds of subsequent psychopathologies, treatments, and/or substances of abuse.

Therefore, high NAA in VS/CN may represent persistent alterations in the neuronal integrity response to the VFD form of aversive early life experience. However, direct evidence linking high NAA with an increased neurotrophic milieu in VS/CN is lacking. That high VS/CN NAA interfaces in the expected direction with other ELS markers—decreased hippocampal volume and increased CSF CRF concentrations—depicts a phenotype entailing parallel long-term alterations,[8] presumably maintained by epigenetic modifications. The findings reported herein may provide a catalyst for further studies in humans, nonhuman primates, and other species regarding a reciprocal hippocampal/NAcc relationship in affective disorders.

Appendix

Table 4. Univariate analyses of general linear model using Log CSF CRF as a predictor variable, group as a categorical variable, and ventral striatum/caudate nucleus NAA values as the repeated measures dependent variable.

		Right				Left			
		ABS NAA		NAA/Cr		ABS NAA		NAA/Cr	
	df	F	p	F	p	F	p	F	p
Group	1	**6.89**	**.022**	0.57	.47	2.55	.14	2.66	.13
Log CRF	1	0.78	.396	0.30	.59	0.32	.58	3.37	.09
Group × log CRF	1	**7.35**	**.019**	0.90	.36	2.60	.13	2.63	.13
Error	12								

Note: An overall side × group × log CSF CRF interaction was observed ($F_{(1,12)} = 8.41$; $p = .013$).
ABS: absolute; NAA: N-acetyl-aspartate, Cr: creatine; CRF: corticotropin releasing-factor.

Table 5. Univariate analyses using left hippocampal volume as a predictor variable, group as a categorical variable, and ventral striatum/caudate nucleus NAA measures as the repeated measures dependent variable.

		Right				Left			
		ABS NAA		NAA/Cr		ABS NAA		NAA/Cr	
	df	F	p	F	p	F	p	F	p
Group	1	0.71	.42	0.36	.56	0.08	.78	**9.65**	**.009**
Left hipp.	1	2.09	.17	1.04	.33	0.48	.50	0.68	.425
Group × left hipp.	1	0.88	.37	0.26	.62	0.07	.80	**9.91**	**.008**
Error	12								

Note: An overall NAA measure × group interaction was observed at a trend level ($F_{(1,12)} = 3.99$; $p = .068$). See Table 1 legend for key. Hipp. = hippocampus; ABS: absolute; NAA: N-acetyl-aspartate, Cr: creatine.

Table 6. Univariate analyses using body mass as a predictor variable, group as a categorical variable, and ventral striatum/caudate nucleus NAA values as the repeated measures dependent variable.

		Right				Left			
		ABS NAA		NAA/Cr		ABS NAA		NAA/Cr	
	df	F	p	F	p	F	p	F	p
Group	1	2.25	.16	0.36	.56	**11.31**	**.005**	0.88	.36
Body mass	1	3.92	.07	1.04	.33	**6.53**	**.024**	0.05	.83
Group × body mass	1	3.07	.10	0.26	.62	**12.24**	**.004**	0.93	.35
Error	12								

Note: An overall rearing group × body mass effect was observed ($F_{(1,13)} = 7.37$; $p = .017$). There was a rearing group × body mass interaction for left VS/CN absolute NAA ($F_{(1,12)} = 12.24$; $p = .004$) reflecting a significant inverse relationship between body mass and left VS/CN absolute NAA in VFD but the absence of inverse relationship in VFD (see Figure 2). ABS: absolute; NAA: N-acetyl-aspartate, Cr: creatine.

Table 7. Univariate analyses using behavioral response as the predictor variable, group as a categorical variable, body mass as a covariate, and ventral striatum/caudate nucleus NAA measures as the repeated measures dependent variable.

		Right				Left			
		ABS NAA		NAA/Cr		ABS NAA		NAA/Cr	
	df	F	p	F	p	F	p	F	p
Group	1	0.17	.690	4.31	.065	0.80	.39	0.59	.46
Body mass	1	4.10	.070	**6.21**	**.032**	0.80	.39	2.98	.12
Timidity	1	0.03	.869	**6.06**	**.034**	0.84	.38	1.07	.33
Group × timidity	1	**11.37**	**.007**	**7.49**	**.021**	1.52	.25	2.81	.12
Group × timidity × mass	1	**7.53**	**.021**	**10.89**	**.008**	0.63	.45	1.96	.19
Error	10								

Note: Since body mass clearly had an influence on VS/CN NAA as a function of rearing, we developed a GLM model controlling for body mass using it as a covariate, behavioral response to a human intruder as a continuous predictor variable and controlling for a body mass × group interactive effect by introducing a triple interaction variable—intruder response × body mass × group. Controlling for body mass effect ($F_{(1,10)} = 6.23$; $p = .032$; greater body mass = less timid), there was a group × behavioral response interactive effect ($F_{(1,10)} = 10.57$; $p = .009$; partial $\eta^2 = 0.51$; see Figure 4). ABS: absolute; NAA: N-acetyl-aspartate, Cr: creatine.

Table 8. Aikake information criterion (AIC), AIC corrected for sample size, Bayesian information criterion, and generalized linear models of interactive terms predicting VS/CN NAA.

	Distribution : NORMAL link function: LOG			
Interactive term predictor variable	Grp × timidity (R ABS NAA)	Grp × weight (L ABS NAA)	Grp × L hipp. (L ABS NAA/Cr)	Grp × log CRF (R ABS NAA)
AIC	−0.67	11.26	4.14	18.67
AICc	11.77	16.72	10.14	24.12
BIC	5.16	15.43	8.00	22.83

Note: The AIC is an estimator of the relative quality of statistical models for a given set of data. Given a variety of models for a data set, AIC provides an estimate of the quality of each model, relative to each of the other models. Thus, AIC provides a means for model selection. Burnham and Anderson (1974) note that, since AICc converges to AIC as N gets large, AICc—rather than AIC—should generally be employed. All interactive effects for the four continuous variables (log CRF, left hippocampal volume, body mass, and timidity response to a human intruder) were significant when using the generalized linear model (see text), which controls for the possibility of nonnormal distributions. AICc values appear to lie between 10.14 and 24.12 (see table for values).

AIC: Akaike information criterion; AICc: AIC corrected for finite sample sizes; BIC: Bayesian information criterion; R: right; L: left; ABS = absolute; Grp = group; NAA: *N*-acetyl-aspartate; Cr: creatine; hipp. = hippocampus; CRF: corticotropin-releasing factor.

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