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Cerebrospinal Fluid Corticotropin-Releasing Factor and Perceived Early-Life Stress in Depressed Patients and Healthy Control Subjects

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Previous studies have reported elevated concentrations of cerebrospinal fluid (CSF) corticotropin-releasing factor (CRF) in patients with major depression. Elevations of CSF CRF have also been reported in adult laboratory animals exposed to the stress of brief maternal deprivation or maternal neglect in the neonatal or preweaning period. The present study was designed to determine whether major depression and a history of perceived early adversity in childhood are independently associated with elevated CSF CRF concentrations in adults. In this case–control study, 27 medication-free adults with major depression and 25 matched controls underwent standardized lumbar puncture for collection of a single CSF sample at 1200. Subjects provided data about significant adverse early-life experiences and rated their global perceived level of stress during pre-school and preteen years on a six-point Likert scale. The mean difference in CSF CRF between depressed patients and controls did not reach statistical significance. In a regression model, perceived early-life stress was a significant predictor of CSF CRF, but depression was not. Perinatal adversity and perceived adversity in the preteen adversity years (ages 6–13 years) were both independently associated with decreasing CSF CRF concentrations. The relationship observed between perceived early-life stress and adult CSF CRF concentrations in this study closely parallels recent preclinical findings. More work is needed to elucidate the critical nature and timing of early events that may be associated with enduring neuroendocrine changes in humans.
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INTRODUCTION

Nearly five decades of research investigating basal and provoked hypothalamic-pituitary–adrenal (HPA) axis activity have established that disturbed regulation of this critical neuroendocrine system is associated with, and perhaps causally related to, depressive disorders. Supra-pituitary-driven hypercortisolemia and impaired glucocorticoid negative feedback inhibition are now considered hallmarks of major depression (Holsboer, 1995). More recently, investigators have been able to examine directly the role of corticotropin-releasing factor (CRF), the principal neuropeptide-regulating stress responsivity, by measuring CRF in cerebrospinal fluid (CSF) and indices of CRF neuronal activity in brain. As CRF serves as the main secretagogue for adrenocorticotropic hormone (ACTH), there has been substantial interest in measuring CSF concentrations of CRF in depressed patients. CRF also acts as an extrahypothalamic neurotransmitter outside of the HPA axis in the limbic system, cerebral cortex, and brainstem. Indeed, several lines of investigation suggest that the vast majority of CRF in CSF is derived from extrahypothalamic sources (Garrick *et al*, 1987; Geracioti *et al*, 1997; Hong *et al*, 1993; Kalin *et al*, 1987). It has been proposed that CRF neurons found in close neuroanatomical proximity to the ventricular system (eg in cortical, limbic, and brainstem regions) contribute the majority CRF to the CSF pool, and may be more relevant to depression and anxiety than those of hypothalamic origin (Post *et al*, 1982).

Most studies in which CRF levels have been measured in human lumbar CSF have shown increased levels of the peptide in depressed patients as compared with non-depressed controls (Banki *et al*, 1987, 1992a, b; France

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et al, 1988; Nemeroff *et al*, 1984; Widerlov *et al*, 1988), although discrepant reports have appeared (Geracioti *et al*, 1997, 1992; Kling *et al*, 1991; Pitts *et al*, 1995; Risch *et al*, 1992; Roy *et al*, 1987). However, secondary analyses in several of these negative studies pointed to higher CSF CRF concentrations in those patients who were dexamethasone suppression test nonsuppressors (Pitts *et al*, 1995; Roy *et al*, 1987) or in those with severe unipolar depression (Risch *et al*, 1992). Data showing normalization of CSF CRF values after successful antidepressant treatment with electroconvulsive therapy (Nemeroff *et al*, 1991; Rudorfer *et al*, 1991) or antidepressant medication (DeBellis *et al*, 1993), and the prediction of sustained long-term antidepressant response following normalization of CSF CRF (Banki *et al*, 1992a, b), support the notion that elevated CSF CRF concentrations reflect a state marker for depressive episodes. Fewer studies have addressed the possibility of persistent CRF dysfunction as a vulnerability trait for depression in humans. Support for the trait hypothesis is derived primarily from a growing preclinical literature, which demonstrates that adverse experiences during early life result in profound and irreversible effects on the mature organism's behavioral and neuroendocrine response to stress (Newport *et al*, 2002a, b).

Separation of rat pups from their mothers during the early postnatal period is one experimental paradigm used to study biological consequences of early stress. As adults, maternally deprived rats have increased anxiety, elevated synthesis, and mRNA expression of CRF in the hypothalamus and amygdala, and decreased responsiveness of the HPA axis to glucocorticoid negative feedback (Ladd *et al*, 1996; Meany *et al*, 1989; Plotsky and Meaney, 1993). An experimental model using unpredictable foraging conditions that leads to alterations in normal maternal–infant interaction has extended the finding of enduring CRF and HPA sequelae to non-human primates (Coplan *et al*, 1996, 2001). The rearing behavior exhibited by variable foraging demand (VFD) mothers is anxious, inconsistent, erratic, and sometimes frankly neglectful, presumably creating a psychological stressor for the infant via perception of 'insecure' attachment with mother (Rosenblum and Paully, 1984). As adults, the offspring of VFD mothers have CSF CRF concentrations that are chronically elevated in comparison with control groups (Coplan *et al*, 1996, 2001).

Recently, both the rodent maternal separation paradigm and the primate VFD model have produced evidence of 'timing effects,' (Mathew *et al*, 2002; Plotsky and Sanchez, 2003). In other words, neuroendocrine data from animals exposed to the experimental stressors at various distinct developmental stages have shown differential directions of abnormalities in HPA function, relative to controls. For example, infant rats exposed to a brief maternal separation stressor during postnatal days 2–14 show robust *increases* in stress-induced HPA hormones in adulthood. However, when the same paradigm is applied to rat pups during postnatal days 5–12, the *opposite* effect is seen, that is, a state of significant HPA *hypoactive response* is observed when the mature animals are stressed (Plotsky, 2003, unpublished data). Similarly, the application of the VFD stressor to mother–infant primate pairs at a later stage of development (starting at age 18–20 weeks *vs* at age 10–12 weeks) essentially *reverses* the direction of the finding of significantly different mean CSF CRF in adulthood (Mathew *et al*, 2002). These and other studies, which include careful analysis of the developmental timing of exposure of young organisms to stress (van Oers *et al*, 1998), suggest that there are distinct and critical periods of brain development during which exposure to stress will impact the direction of enduring regulatory shift of the HPA axis.

The translation of hypotheses generated by such preclinical studies to human subjects is inherently challenging. Experimental designs frequently must rely on retrospective reporting of remote adverse events that are difficult to validate and quantify objectively. Breier *et al*, 1988 recruited subjects with a history of parental separation and found that a high proportion fulfilled the criteria for adult psychiatric disorders. Furthermore, the subgroup with a psychiatric history had higher basal cortisol concentrations than those with no such history or controls. Heim *et al* (2001, 2000, 2002) conducted clinical neuroendocrine investigations of the relationship between childhood trauma, adult depression, and HPA function. Plasma ACTH and cortisol responses of female subjects with depression, with and without early adverse life events, were examined in a standardized human psychosocial stress paradigm. Early-life trauma was associated with enhanced ACTH responsivity, and the participants with both depression and a history of early-life trauma had elevated responses of both ACTH and cortisol (Heim *et al*, 2000). These subjects subsequently took part in a standardized CRF stimulation test (Heim *et al*, 2001). A self-reported history of childhood trauma, rather than a diagnosis of major depression, conferred the greatest degree of HPA axis dysregulation. Multiple regression analysis revealed that a history of childhood abuse *per se* was related to increased neuroendocrine stress hyperreactivity, and that the interaction between childhood abuse and adulthood trauma was the most powerful predictor of ACTH responsiveness (Heim *et al*, 2002).

Our study examined the relationship between self-reported early-life adversity, depression, and CSF CRF concentrations in a sample of adult depressed patients and nondepressed controls. Based on the preclinical and clinical literature, it was hypothesized that both the presence of major depressive illness and subjective reports of significant perceived early-life adversity would predict adult levels of CSF CRF.

SUBJECTS AND METHODS

In all, 27 adult outpatients fulfilling the DSM-IV criteria for unipolar major depressive disorder (MDD) and 25 healthy control subjects, selected to age and gender match the patient group, provided voluntary written informed consent to participate in this study. The protocol was approved by the institutional review boards of Yale University (New Haven, CT, USA) and Butler Hospital (Providence, RI, USA), and conducted at both institutions. Healthy subjects were recruited by postings around college/university campuses and on community bulletin boards. Depressed patients were recruited from those who presented seeking treatment in one of several clinical research trials for major depression. While several different versions of the Hamilton Depression Rating Scale (Hamilton, 1960) were used to

determine eligibility for the clinical trials, all required the equivalent of a baseline score greater than 17. Semistructured diagnostic interviews were used to determine the presence of unipolar MDD (patient group) or the absence of any current and lifetime DSM-IV Axis I disorder (controls). Individuals with any other major Axis I comorbidity were excluded. While Axis II disorders were not formally assessed, those patients who presented with symptoms or characteristics suggestive of prominent personality pathology were excluded from entry into the CSF and depression treatment protocols. Also excluded were subjects with any clinically significant medical disorder, based on history, physical examination, and laboratory studies, which included a complete blood count, serum chemistries, liver and thyroid function tests, urine toxicology, electrocardiogram, and pregnancy test for women. All subjects were medication free for at least 2 weeks by the time of participation and were remunerated for participating. Depressed subjects underwent CSF sampling within 2 weeks prior to starting their clinical trial antidepressant treatment.

Subjects underwent a single standardized lumbar puncture (LP) and subsequently completed a qualitative questionnaire about early-life experiences. The questionnaire included items with face neutrality (eg gestational, neonatal, and childhood medical history; birth order; moving residence during childhood; family composition), as well as some items that more obviously assessed emotionally difficult childhood experiences (eg death of a close loved one; perceived parental availability; recalled physical, sexual, or emotional abuse; witnessing violence; parental substance abuse; family discord). Other items asked about perceived stress in childhood related to the subject's race, ethnic background, sexual orientation, or social status. Subjects were then asked to rate their perceived level of overall stress during pre-school years (ages birth–5 years) and preteen years (ages 6–13 years) on a six-point Likert scale, relative to what they considered normal for their peer cohort (global perceived early-life stress, GPELS). Subjects were aware that the study was intended to examine biological differences between depressed patients and healthy controls, but were not given further details about how demographic and historical information would be analyzed. Adverse early-life experiences were not discussed with subjects during the screening interviews or at any point during the LP.

All LP procedures were standardized to yield CSF samples at 1200 ± 15 min. All subjects agreed to comply with instructions for a modified diet and activity schedule in the 24 h preceding the LP. Specific measures were taken to reduce anxiety and HPA axis arousal associated with the LP procedure. Subjects did not disrobe or change into hospital gowns for the procedure, but wore loose clothes that were simply rolled away from the lumbar area. Subjects did not enter the procedure room until immediately before the LP began, and medical supplies such as needles and equipment used in the LP were kept out of subjects' view throughout the protocol. Low-volume soothing background music was played, and visually interesting posters decorated the wall before the subjects, who were placed in a comfortable leaning-forward seated position on a bed. Subjects were repeatedly encouraged to give feedback about their comfort so the physician could achieve a relatively pain-free LP through the use of comfortable positioning and liberal application of local anesthetic. Casual conversation between the subject and physician performing the LP continued throughout the procedure. If the procedure was not successful in producing a CSF sample by 30 min after the start of sterile preparations, the procedure was terminated.

Following the intradermal injection of 1% lidocaine, a 20-gauge introducer needle was used to penetrate skin and superficial tissue. The introducer is necessary because the Sprotte® 24-gauge pencil point spinal needle is very thin and has a relatively dull tip. The spinal needle was then inserted, through the introducer, to the L4–L5 interspace. A total of 12 cc of CSF was collected, immediately divided into 0.5 ml aliquots, and frozen at -80°C until assayed. Concentrations of CSF were measured by radioimmunoassay in duplicate using an antiserum (oC33) raised in rabbits against ovine CRF that was generously provided by Wylie Vale, Ph.D., of the Salk Institute (Banki *et al*, 1987).

In the initial statistical analyses, *t*-tests and χ^2 tests were used to compare continuous measures (ie CSF CRF concentrations, age, GPELS values) and categorical variables (ie gender, dichotomous ratings on GPELS), respectively, between depressed patients and healthy controls. Pearson's correlation coefficients were calculated for relevant continuous variables. One subject did not complete the pre-school and preteen GPELS ratings and was excluded from analyses of these variables. Self-reported perinatal history was derived from the questionnaire items, and perinatal stress was scored as present if the subject reported having been the product of a medically complicated pregnancy or delivery (eg premature birth, nuchal cord, low birthweight), or if there was a prolonged hospital stay for neonatal care for any other reason. As the psychometric properties of the questionnaire have not been fully established, individual items describing other types of early-life stressors were not examined statistically with regard to CSF CRF concentrations.

Multiple regression analysis with forced entry was performed to determine the predictive value of four independent variables on adult CSF CRF concentrations: diagnosis (depressed *vs* control), pre-school GPELS (1–6), preteen GPELS (1–6), and 'perinatal stress' rating (present *vs* absent). All tests were two-tailed and considered significant at $p < 0.05$.

Depression rating scale total scores were not available for enough patients to allow for the analysis of the relationships between severity of illness other key variables. In light of the potential bias introduced by depressed mood at the time of reporting early-life events, secondary analyses were conducted on the sample of healthy controls alone ($n = 25$). Pearson's correlation coefficients were determined for examination of the relationships between CSF CRF and the four key variables, and partial correlation coefficients were calculated for pre-school and preteen GPELS analyses. The small sample size precluded meaningful application of a regression analysis in this subgroup.

RESULTS

Depressed patients and healthy control subjects were similar with regard to age and sex (Table 1). Mean CSF

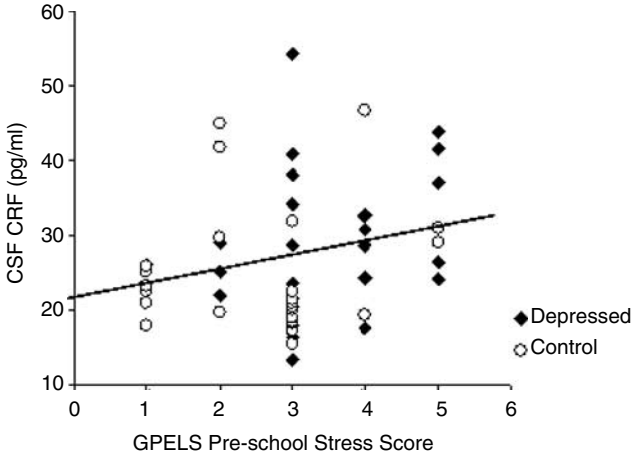

Figure 1 Relationship between CSF CRF and perceived pre-school stress rating.

CRF concentrations were higher among depressed patients, but the difference between groups did not reach statistical significance (mean ± SD, 29.0 ± 9.4 *vs* 24.9 ± 8.6 pg/ml; $t = 1.6$, df = 50, $p = 0.106$). A similar proportion of each subject group endorsed the criteria for perinatal stress: 19% ($n = 5$) of depressives and 16% ($n = 4$) of controls. Mean GPELS scores of perceived early-life stress during the pre-school years were significantly higher for the group of depressed patients as compared with the controls (3.4 ± 1.1 *vs* 2.6 ± 1.2; $t = 2.6$, df = 49, $p = 0.012$). Nearly half (42%) of the depressed subjects rated themselves as having had a 'higher than average' degree of exposure to stress during their childhood from birth through age 5 years, yet only four (16%) of the controls perceived a high level of stress relative to their peers during that period ($\chi^2 = 4.2$, $p = 0.04$). The two groups did not differ statistically with regard to GPELS ratings of stress during the preteen years (4.1 ± 1.2 *vs* 3.5 ± 1.3; $t = 1.639$, df = 49, $p = 0.108$); nine (53%) of depressed subjects and nine (48%) of controls rated themselves as having experienced above average levels of stress during ages 6–13 years. Scores on the pre-school and preteen GPELS correlated significantly with each other ($r = 0.62$, $p < 0.001$), reflecting the fact that many subjects endorsed a consistent degree of perceived adversity across their childhood and latency years.

CSF CRF values for the pooled (depressed and control) subjects ranged from 13.4 to 54.1 pg/ml, with a median value of 24.1 pg/ml and mean ± SD of 27.06 ± 9.18 pg/ml (Figure 1). Relationships between perceived early-life stress ratings and CSF CRF concentrations for the pooled groups were analyzed in a regression model that contained the four predictor variables and accounted for 22% of the variance in CSF CRF concentration (F = 3.3; df = 4, $p = 0.018$) (Table 2). Pre-school GPELS score was a significant *positive* predictor ($\beta = 0.438$, $p = 0.015$) of CSF CRF concentration in this model. Preteen GPELS score emerged as a significant *negative* predictor of CSF CRF concentration ($\beta = -0.385$, $p = 0.029$), as did perinatal stress ($\beta = -0.317$, $p = 0.023$). The presence of adult major depression was not a significant predictor of CSF CRF concentrations ($\beta = 0.165$, $p = 0.241$).

Table 1 Comparison of Subjects Based on the Presence of Major Depression

	Depressed ($n = 27$)	Controls ($n = 25$)	*p*-value
Age (mean ± SD) (years)	37.4 ± 13.0	34.0 ± 11.5	NS
Gender (% (*n*))	—	—	NS
Male	41% (11)	48% (12)	
Female	59% (16)	52% (13)	
CSF CRF (mean pg/ml ± SD)	29.0 ± 9.4	24.9 ± 8.5	0.106
Perinatal stress			
(Any pregnancy/obstetrical/ neonatal complications) *n* (%)	5 (19%)	4 (16%)	NS
GPELS (global perceived early-life stress)			
Pre-school years (ages birth–5 years) rating			
Score (mean ± SD)[a]	3.4 ± 1.1	2.6 ± 1.2	0.012
'High' categorical rating (*n* (%))[b]	11 (42%)	4 (16%)	0.039
Preteen years (ages 6–13 years) rating			
Score (mean ± SD)[a]	4.1 ± 1.2	3.5 ± 1.3	0.108
'High' categorical rating (*n* (%))[b]	9 (53%)	8 (47%)	NS

[a]Six-point Likert scale where 1 = least stress to 6 = most stress.
[b]Dichotomization of GPELS ratings: 'high' stress defined as 'more stressful than most kids', 'extremely stressful', or 'the most stress I have ever known;' 'low' stress defined as 'essentially stress free', 'less stress than most kids', or 'about average'.
NS = nonsignificant.

Table 2 Multiple Linear Regression Analysis: Predictors of CSF CRF in Adults

Predictor	Relationship to CRF	β[b]	*p*-value
Pre-school stress (GPELS ages 0–5 years)[a]	Positive	0.438	0.015
Perinatal stress (present *vs* absent) (pregnancy/obstetrical/ neonatal complications)	Negative	−0.317	0.023
Preteen stress (GPELS ages 6–13 years)[a]	Negative	−0.385	0.029
Current major depression	Positive	0.165	0.241

[a]Six-point Likert scale where 1 = least stress to 6 = most stress.
[b]Standardized regression coefficient.

Data from the 25 healthy control subjects were examined independently to remove possible biases from self-reporting perceived early-life adversity while in a depressed state. Among healthy controls, perceived levels of stress during the pre-school years (birth–age 5 years) were associated with elevated CSF CRF concentrations at a trend level ($r = 0.34$, $p = 0.09$) and perceived levels of stress during the latency developmental period (ages 6–13 years) were significantly associated with CSF CRF concentrations in the *opposite* direction ($r = -0.42$, $p = 0.04$). Healthy

subjects' age and reports of perinatal adversity showed no relationship with CSF CRF concentrations.

DISCUSSION

Consistent with the mixed findings with respect to CSF CRF in major depression described in the literature, we found that CSF CRF concentrations were somewhat higher in a group of medication-free depressed adults than in non-depressed healthy controls, but this difference fell short of statistical significance. The notion that depression is independently associated with CRF hyperactivity was also not supported by the results of our regression analysis, where the presence of depression failed to predict CSF CRF concentrations. Rather, the data suggest that perceived early-life adversity, commonly reported among depressed patients, may be a mediating variable between depression and adult CSF CRF concentrations, and that the timing of exposure to adversity may impact the direction of HPA regulation shift. Assessments of (perceived or observed) early-life stress have not been included in previously published reports describing elevated CSF CRF concentrations among depressed samples.

We found perceived levels of adversity in different stages of development to be associated with adult CSF CRF concentrations, independent of major depression. In the pooled subjects group, global self-ratings of stress during pre-school years emerged as a significant *positive* predictor of adult CSF CRF concentration. This finding is consistent with the growing body of preclinical research, suggesting that exposure to stress during critical early developmental periods induces enduring upregulation in HPA function, often indicated by elevated CSF CRF concentrations, which may represent a biological vulnerability for depression, anxiety, or other pathology in adulthood. We are aware of one other clinical research group that recently presented preliminary data showing a similar positive relationship between self-reported childhood trauma scores and CSF CRF concentrations in adult males with personality disorders (Lee *et al*, 2002).

Perhaps, more intriguing is our finding that perceived early-life stress was associated with a relatively increased or decreased CSF CRF concentrations depending on the reported developmental timing of the perceived stress exposure (Table 2). Whereas stress before age 6 years was associated with elevated CSF CRF concentrations, both perinatal stress and preteen stress were predictive of *lower* CRF concentrations. A pattern of hypoactive HPA responsivity associated with perinatal stress was also suggested by results of a study of 8–14 year olds in whom a history of premature birth was associated with attenuated salivary cortisol and heart rate responses to a standardized psychosocial stressor in comparison to full-term subjects (Buske-Kirschbaum *et al*, 2000). Similarly, in preclinical work, prenatal immune challenge has been found to diminish HPA axis reactivity in adult rats (Reul *et al*, 1994). Other preclinical investigations have revealed developmental effects similar to those found in the present study. A recent study of non-human primates reared in the VFD stress paradigm shifted the age of exposure to VFD rearing from that of the previous studies. When VFD rearing began with infants at 18 weeks of age, the results were significantly *lower* CSF CRF concentrations compared to control primates, whereas prior studies of VFD exposure beginning at 10–12 weeks revealed *increases* in CSF CRF compared to controls (Mathew *et al*, 2002). There is also some evidence of critical periods for HPA axis sensitivity to environmental stimuli in rodents, with heightened sensitivity in the first week of life and again after weaning (Meany *et al*, 1996; Plotsky *et al*, unpublished observations).

The present findings may also be consistent with a hypothesis recently advanced by Yehuda *et al* (2001) that early childhood stressors may be more likely to predispose to the types of HPA axis changes typically seen with depression, whereas stressors occurring later in life may lead to neuroendocrine supersuppression and PTSD. Support for the hypothesis that earlier stressors are associated with a neuroendocrine pattern characteristic of depression is provided by a study by Petitto *et al* (1992), who found that among mood disorder patients with a history of parental or sibling loss before the age of 20 years, age at first loss was strongly and negatively correlated with postdexamethasone cortisol levels. These different lines of investigation support the notion that the timing of stress exposure may be a critical determinant of enduring neuroendocrine stress responsivity. Additional research into the neuroendocrine and behavioral effects of stressors with regard to the quality, intensity, and timing of early-life adversity is needed for a more complete understanding of the complex relationship between stress, HPA axis function, and psychopathology.

Our findings are limited in some ways, which deserve consideration. A retrospective, self-report method was used to capture the nature and perceived intensity of early-life stressors. Occurrence of the stressors identified by our qualitative questionnaire and the GPELS scores were not independently confirmed. These measures reflected perceived and recalled experiences, which may not be as strongly associated with enduring HPA disturbances as stressors that are experimentally standardized or 'objectively' quantified.

Retrospective assessment of early-life stress is controversial and currently a topic of much consideration among researchers. Without benefit of a subject cohort prospectively and continuously followed from birth (and perhaps prenatally) with close attention to even subtle environmental influences throughout development, it is impossible to conclude that one has access to valid and complete information about early-life adversity. Even when interview assessments of stress are conducted in 'prospectively' followed cohorts at points in close temporal proximity to exposure to objectively defined adverse conditions, the subject's report necessarily reflects his or her gestalt of discrete events, context, and unique interpretations. Unless biological data (eg vitals signs evidence of autonomic nervous system arousal, acute cortisol release) are available at the time of exposure to a stressor, investigators must rely on subjects' perceptions and reports of how much stress they experienced.

Clinical investigations into the relationship between adverse developmental environments and adult health outcomes are often considered limited by the use of self-

report data. While prospectively observed and objectively validated life events might generally be considered the most scientifically valid, perceived reality may be the more relevant determinant of health outcomes when it comes to stress biology. As many limbic and higher cortical brain structures provide input to the hypothalamus during the interpretation of a stimulus as threatening, there will be considerable individual variability in the degree of HPA axis arousal produced by any given stimulus. At least among healthy individuals, one's subjective, uniquely integrated *perception* of severity of stress probably 'counts' more than how stressful the experience is objectively rated by external standards. Perhaps more problematic is the fact that such perceptions may be distorted when recall of childhood adversity is conducted in adult individuals in an acutely depressed state. Indeed, when compared with our healthy subjects, depressed individuals were significantly more likely to rate their early lives as highly stressful. We cannot conclude whether such a finding simply represents recall biases due to prominent depressive cognitions, or whether it reflects the fact that individuals exposed to stress are at a greater risk of developing depression in adulthood. However, the replication of some of the significant relationships we found between CSF CRF concentrations and perceived early-life stress in a sizable group of healthy controls makes it less likely that our results are attributable to that potential confound. Serial assessment of perceived early-life stress in a depressed cohort during various clinical states (eg depressed, partially remitted, fully remitted, relapsed) would help elucidate if and how depression impacts the reporting of early-life adversity.

The GPELS, a simple Likert self-rating of *global* perceived stress in two distinct time periods, is not a validated measure. We developed the GPELS to address the fact that available, psychometrically established instruments of early-life trauma were insufficiently sensitive to many types of events which subjects have anecdotally described as highly stressful. No published scale incorporates the breadth of items (eg perinatal health, perceived stress due to race, sexual identity, or ethnic group) that we thought might be relevant in an investigation of CSF CRF concentrations. For example, recent research in rodents has shown that exposure to bacterial endotoxin during the neonatal period permanently alters the development of the HPA axis, resulting in hypersecretion of corticosterone after subsequent stress exposure even in adulthood (Hodgson *et al*, 2001; Shanks *et al*, 2000). This underscores the need to assess a broad range of early-life stressors, including physically as well as psychologically distressing experiences. Any perceived or actual threat to an organism's integrity and survival has the potential to stimulate CRF release and initiate a multisystem stress response in the service of self-protection and self-preservation. While sexual and physical abuse have consistently been identified as early-life stressors related to adult psychopathology (Browne and Finkelhor, 1986), the psychiatric and neuroendocrine consequences of other types of stressors have not been systematically studied in humans. Given the practical and ethical limitations of exposing human neonates and young children to experimentally manipulated adverse experiences, future studies in this area will likely continue to be characterized by a lack of standardization of stressors and limited ability to control potentially confounding variables. Whether recalled severity of global adversity reflects a valid and reliable summary of overall stress burden during a period of time remains to be explored in future studies. However, its possible utility is supported by results of a recent study showing that retrospective, self-ratings of global childhood victimization, rather than objective, externally validated data with respect to specific incidents of childhood abuse, were associated with the development of unexplained pain syndromes in adulthood (Raphael *et al*, 2001). Another example providing validation for the use of self-report assessment of global perceived stress for predicting important health outcomes comes from 35-year follow-up data on healthy undergraduate students who participated in the Harvard Stress Mastery Study (Russek and Schwartz, 1997a, b; Russek *et al*, 1998). Those describing themselves as exposed to low levels of parental caring were found to have a four-fold greater risk of suffering from chronic illnesses such as coronary artery disease, hypertension, duodenal ulcer, alcoholism, and depression in midlife. To ensure that the extraneous state and trait variables are not shaping the reporting styles of participants, future studies that involve global self-ratings or measures of perceived stress might also include assessments of adult 'temperament' or neuroticism measures, as well as indices of state and trait anxiety.

Research investigating the neurobiological consequences of early-life adversity in human samples poses considerable methodological challenges, and also holds great promise for the detection of biological risk factors and the prevention of psychiatric illness. Findings from such studies, in conjunction with the recent emergence of drugs that target glucocorticoids and CRF receptors in the adult brain, could lead to effective prophylaxis or treatment of psychiatric symptoms in individuals who have been exposed to early-life adversity.

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