Cerebrospinal Fluid Corticotropin-Releasing Hormone Levels Are Elevated in Monkeys with Patterns of Brain Activity Associated with Fearful Temperament

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Background: *Asymmetric patterns of frontal brain activity and brain corticotropin-releasing hormone (CRH) systems have both been separately implicated in the processing of normal and abnormal emotional responses. Previous studies in rhesus monkeys demonstrated that individuals with extreme right frontal asymmetric brain electrical activity have high levels of trait-like fearful behavior and increased plasma cortisol concentrations.*

Methods: *In this study we assessed cerebrospinal fluid (CSF) CRH concentrations in monkeys with extreme left and extreme right frontal brain electrical activity. CSF was repeatedly collected at 4, 8, 14, 40, and 52 months of age.*

Results: *Monkeys with extreme right frontal brain activity had increased CSF CRH concentrations at all ages measured. In addition, individual differences in CSF CRH concentrations were stable from 4 to 52 months of age.*

Conclusions: *These findings suggest that, in primates, the fearful endophenotype is characterized by increased fearful behavior, a specific pattern of frontal electrical activity, increased pituitary–adrenal activity, and increased activity of brain CRH systems. Data from other preclinical studies suggests that the increased brain CRH activity may underlie the behavioral and physiological characteristics of fearful endophenotype.* Biol Psychiatry 2000;47: 579–585 © *2000 Society of Biological Psychiatry*

Key Words: CRH, monkeys, fear, temperament, brain activity

Introduction

Rhesus monkeys and humans exhibit similarities in fear-related behavioral responses (Kalin et al 1989), hypothalamic–pituitary–adrenal (HPA) activity (Lyons et al 1999), and patterns of brain electrical activity (Davidson et al 1992; Davidson et al 1993). Both species also exhibit individual differences in their propensity to engage in fearful behaviors (Kagan et al 1988; Kalin et al 1989). Thus, rhesus monkeys are an excellent species in which to investigate the biological underpinnings of anxiety, depression, and fearful temperament (Kalin and Shelton 1989). Assessing asymmetric patterns of electrical activity from frontal brain regions is particularly informative in understanding individual differences in emotionality. In rhesus monkeys, individual differences in asymmetric frontal electrical activity remain stable over time, suggesting that this is a trait characteristic (Kalin et al 1998a). Compared to monkeys with extreme left frontal activity, those with relative right frontal activity exhibit more fear-related behaviors, such as freezing and defensive hostility (Kalin et al 1998a). Animals with extreme asymmetric right frontal activity also have elevated basal plasma levels of the stress hormone, cortisol (Kalin et al 1998a). In addition, individual differences in freezing are correlated with baseline cortisol concentrations (Kalin et al 1998b). These findings are relevant to understanding psychopathology, because studies in humans also demonstrate stability in individual differences in the degree of asymmetric frontal electrical activity (Tomarken et al 1992), and humans with asymmetric right frontal activity tend to have dispositionally negative affect. In addition, extreme right frontal asymmetric electrical activity has been associated with anxiety and depressive disorders (Davidson 1995).

Corticotropin-releasing hormone (CRH) is a neuropeptide that is fundamental in mediating the stress response and is hypothesized to play a mechanistic role in mediating fear, anxiety, and depression (De Souza 1995; Vale et al 1981). CRH-containing neurons and CRH receptors are found throughout numerous brain regions (De Souza et al 1985; Swanson et al 1983) and CRH-containing neurons located in the hypothalamic paraventricular nucleus (PVN) are largely responsible for stress-induced activation of the peripheral pituitary–adrenal system (Plotsky et al 1984; Swanson et al 1983). CRH neurons and receptors located in the limbic structures, brain stem, and cortex are important in mediating the autonomic and behavioral aspects of the stress response (Butler et al 1990; Curtis et al 1997; Swiergiel et al 1992). In primate and human studies, cerebrospinal fluid (CSF) concentrations of CRH are used as an indirect assessment of the activity of brain

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CRH systems (Kalin et al 1987; Nemeroff et al 1984), and data suggest that CSF concentrations reflect activity of brain CRH systems in areas other than the PVN (Kalin et al 1987).

Clinical research indicates that some, but not all, individuals with depression have increased CSF CRH concentrations (Banki et al 1987; Geracioti et al 1997; Nemeroff et al 1984), which normalize with recovery (Banki et al 1992; Nemeroff et al 1991). Data from patients with anxiety disorders are less consistent, but increased CSF CRH levels have been reported in some individuals with obsessive-compulsive and post-traumatic stress disorder (Altemus et al 1992; Bremner et al 1997). Data from postmortem studies of brains from depressed suicide victims support the contention that increased CSF CRH levels reflect increased activity of brain CRH systems. In addition to elevated CSF CRH levels, depressed suicide victims have increased PVN CRH content and CRH mRNA (Raadsheer et al 1994, 1995), and a decrease in CRH receptors in frontal cortex (Nemeroff et al 1988). The decrease in frontal cortex CRH receptors is believed to be a compensatory response to increased availability of CRH in cortical regions.

The purpose of the present study was to further characterize the biological characteristics of monkeys with extreme asymmetric right frontal electrical activity by assessing CSF CRH levels. In addition to increased fearfulness and elevated plasma cortisol concentrations, we hypothesized that monkeys with extreme right frontal activity would have increased CSF CRH levels. Elevated CSF CRH in these animals would support the hypothesis that increased brain CRH activity is a mechanism underlying the behavioral and physiological characteristics associated with fearful temperament.

Methods and Materials

Animals

In 50 rhesus monkeys *(Macaca mulatta)*, regional electroencephalography (EEG) was collected at approximately 13 months of age (mean = 13.1 months; range = 9.7–20.2 months) and extreme animals were selected based on extreme left ($n = 10$; 5 females and 5 males) and extreme right asymmetric frontal EEG patterns ($n = 9$; 3 females and 6 males) (Kalin et al 1998a). The EEG data from these monkeys was previously reported (Kalin et al 1998a). In the extreme animals, fear-related behavior was assessed, and blood and CSF were sampled at approximately 4, 8, 14, 40, and 52 months of age (at 52 months of age two CSF samples were collected). In the 4-month age group ($n = 14$) CSF CRH data were available from a subset of the extreme left frontal ($n = 7$; 3 females, 4 males) and extreme right frontal group ($n = 7$; 2 females and 5 males). In the 40-month age group ($n = 18$), CSF CRH data were unavailable from one female in the left frontal group, and in the 52-months age group ($n = 17$), the left group was comprised of 4 females and 4 males, and the right group had 6 females and 3 males. The exact ages when CSF was collected were as follows: 4-month group ($\bar{X}$ = 3.9 months; range = 3.2–4.8 months), 8-month group ($\bar{X}$ = 7.9 months; range = 7.2–8.8 months), 14-month group ($\bar{X}$ = 14.3 months; range = 10.6–20.9 months), 40-month group ($\bar{X}$ = 40.3 months; range = 37.2–46.4 months), and 52-month group ($\bar{X}$ = 52.1 months; range = 48.6–59.0 months). All animals were maintained on a 12-hour light–dark cycle (lights on 0600 to 1800 hours) and were housed at the Wisconsin Regional Primate Center and the Harlow Primate Laboratory. Animal housing and experimental procedures were in accordance with institutional guidelines. The plasma cortisol and behavioral data from these animals was previously reported (Kalin et al 1998a).

Sampling Procedures

As rapidly as possible after the animals were anesthetized with ketamine 10 mg/kg IM, blood was sampled by femoral venipuncture and two mL of CSF was obtained by percutaneous puncture of the cisterna magna (Kalin et al 1987). All samples were obtained between 0830 and 1030 hours to control for the diurnal variation in CSF CRH (Kalin et al 1987). CSF samples were obtained an average of 8 min after approaching the cage. This 8 min included the time elapsed from ketamine administration to the time of sampling, which averaged 7 min. CSF samples were stored at −70°C until assayed.

CRH Radioimmunoassay

Immunoreactive CRH was measured in 100 μL of CSF with an antiserum developed in rabbits against CRH, coupled to human α-globulins with bisdiazotized benzidine (IgG Corp., Nashville, TN). The antiserum is directed against the N-terminal portion (antibody recognition site hCRH (4–20)) of the intact peptide and has the following molar cross reactivities: oCRH, 100%; urocortin ~25%; oCRH(1–20), 100%; hCRH(4–41), 100%; oCRH(1–12), <0.1%; hCRH(28–41), <0.1%; hCRH(38–41) <0.1%; and hCRH(6–33), 1.0%. It does not cross-react with adrenocorticotropic hormone, α-lipotropin, β-lipotropin or β-endorphin. The detection limit, defined as the ED90, was 0.89 pg/tube. The intraassay and interassay coefficients of variation were 6.1% and 10.2%, respectively (Kalin et al 1987). All samples obtained at 4, 8, and 14 months were analyzed in the same assay. Samples obtained at 40 and 52 months were analyzed in separate assays.

EEG Recording and Quantification

To collect EEG data, animals were manually restrained, left and right frontal, parietal, and mastoid gold cup electrodes were placed according to the standard 10/20 system. The five active leads (left and right frontal and parietal and right mastoid) were referenced to the left mastoid and the linked mastoids were mathematically derived. All electrode impedances were below 5,000 ohms. We used a mathematically derived averaged mastoid reference rather than a physically linked mastoid reference

because of the possible contribution of slight impedance differences between the mastoids affecting the effective spatial location of the reference when they are physically linked. When an averaged mastoid reference is mathematically derived, the data are recorded referenced to a single mastoid. The other mastoid is then recorded as an active channel. In this way, the high-input impedance of the amplifiers prevent any slight difference in electrode impedances from affecting the recorded signal at the scalp surface. This is the preferred method for quantitative EEG analysis when asymmetric effects are the subject of analysis. EEG was amplified using Grass Model 12 EEG amplifiers, with a gain of 20,000 and a band-pass of .1 to 200 Hz. A minimum of 20 artifact-free seconds of EEG was required and we sampled a mean of 70.95 artifact-free seconds of EEG per subject.

EEG signals were passed through active anti-aliasing low-pass filters set at 210 Hz with a 36 dB/octave roll-off. The output of the filters was digitized on-line at 500 Hz with an 80486 PC-clone, equipped with a 12-bit, 32-channel A/D board and signal acquisition software and stored on magnetic tape cartridges.

All data were edited for artifact on a high-resolution graphics monitor. EEG from each of the scalp leads during all artifact-free periods was analyzed. Epochs of EEG 2.00 sec in duration were extracted through a Hamming window. A Fast Fourier Transform (FFT) was applied to each chunk of EEG, with epochs overlapping by 50%. The FFT output was in μV^2. Data were aggregated into band power for the 4–8 Hz band and expressed as μV^2/Hz based upon our previous research with rhesus monkeys (Davidson et al 1992, 1993). EEG data were log-transformed to normalize the distribution of power values. For each animal, asymmetry scores were calculated by subtracting the left from the right frontal power. Because power is inversely related to activation, lower activation asymmetry scores indicate greater relative right frontal activation.

Monkeys with EEG asymmetry scores based on activation of .7 standard deviations greater than the mean were classified as extreme left frontal and those with asymmetry scores of .7 standard deviations less than the mean were classified as extreme right frontal (Davidson 1998).

Data Analysis

To test whether CSF CRH levels were stable within individuals, simple regression analyses were performed on CSF CRH concentrations between each successive age, as well as between the youngest (4 months) and oldest (52 months) ages sampled. Because two CSF samples were collected in the 52-month group, the mean of the two concentrations was used. Because the actual age ranged within each age group, regression analyses were also performed covarying for age.

A *t* test was performed to establish that the EEG asymmetry scores for the right and left frontal groups were significantly different. To examine whether subjects with extreme right frontal asymmetry scores had higher CSF CRH levels than extreme left frontal animals, a repeated-measures analysis of variance (ANOVA) was performed followed by post-hoc *t* tests at each age. The overall mean CRH concentration was used in cases of missing data points. Additional *t* test were performed at each age to assess whether CSF CRH concentrations differed by sex. To exclude the possibility that sampling procedures affected CSF CRH concentrations, regression analyses were performed at each age between CSF CRH concentrations and the total time to sample CSF, as well as the amount of time elapsed after administration of anesthesia until the CSF sample was obtained. In addition, *t* tests were performed at each age to compare the total time to sample between the left and right frontal groups.

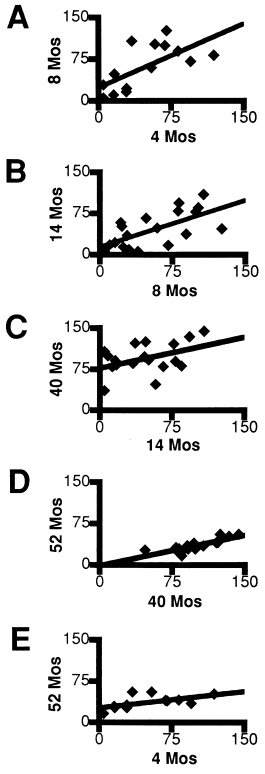

Figure 1. Stability of CSF CRH concentrations from: **(A)** 4–8 months, $r = .669$, $p < .01$; **(B)** 8–14 months, $r = .656$, $p < .01$; **(C)** 14–40 months, $r = .437$, $p < .05$; **(D)** 40–52 months, $r = .827$, $p < .001$; **(E)** 4–52 months, $r = .583$, $p < .05$.

Results

As can be seen in Figure 1, CSF CRH concentrations remained stable over time: 4–8 months ($n = 14$; $r = .669$; $p < .01$), 8–14 months ($n = 19$; $r = .656$; $p < .01$), 14–40 months ($n = 19$; $r = .437$; $p < .05$ one-tailed), 40–52 months ($n = 16$; $r = .827$; $p < .001$), and 4–52 months ($n = 12$; $r = .583$; $p < .05$). Analyses covarying for age did not significantly differ from the simple correlations. In addition, at each age CSF CRH concentrations were not significantly correlated with the total time to collect the sample, nor with the time from anesthesia administration. At 4, 8, 40, and 52 months no significant gender differences in CRH concentrations were

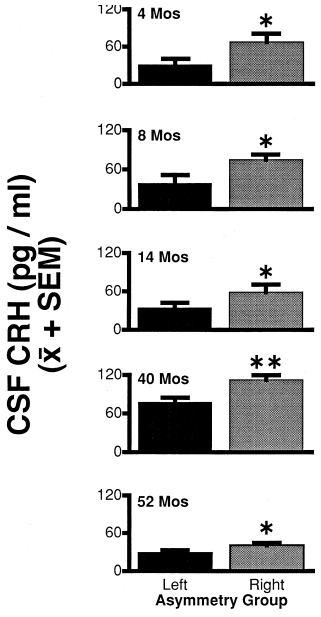

Figure 2. CSF CRH concentrations in the extreme left compared to the extreme right frontal animals at 4, 8, 14, 40, and 52 months of age. $^{**}p < .01$, $^{*}p < .05$.

found; however at 14 months males had higher CRH levels (see below).

The mean frontal EEG asymmetry scores for the extreme left and right frontal animals as defined by EEG at 13 months of age significantly differed from each other ($t = 9.50$; df = 17; $p < .0000001$), and were respectively 0.227 and −0.243. No significant difference in the asymmetry scores was found comparing male to females. Repeated measures ANOVA revealed that across all ages extreme right frontal animals had significantly elevated CSF CRH concentrations ($F = 10.31$; df = 1,17; $p < .006$). Post-hoc t tests showed that extreme right frontal monkeys had significantly elevated CSF CRH concentrations at each age sampled: 4 months ($t = 2.24$; df = 12; $p < .05$), 8 months ($t = 2.36$, df = 17, $p < .03$), 14 months ($t = 1.78$, df = 17, $p < .05$ one-tailed), 40 months ($t = 3.37$, df = 17; $p < .004$), and 52 months ($t = 2.78$, df = 15, $p < .015$). At 14 months males had higher CRH levels compared to females ($t = 2.11$; df = 17; $p < .05$) and at this age males constituted 5/10 of the left frontal group and 6/9 of the right frontal group (Figure 2).

Discussion

These data demonstrate that individual differences in monkeys' CSF CRH concentrations sampled during nonstressful conditions are stable from the age of 4 to 52 months. This is of interest, because we have also found that an animal's propensity to engage in fearful behaviors, such as freezing, and its degree of asymmetric frontal electrical activity, are also stable over time (Kalin et al 1989, 1998a). These findings suggest that, like freezing and asymmetric frontal electrical activity, CSF CRH concentrations are a trait-like variable. To the extent that CSF CRH concentrations reflect activity of brain CRH systems, this finding suggests that individual differences in brain CRH activity are also stable. It is possible that the differences in CSF CRH concentrations between right and left frontal groups could be due to an interaction between an animal's fearful disposition and the stress associated with sampling. We believe this is unlikely, because we previously showed that acute stress does not alter CSF CRH concentrations (Kalin et al 1987); however, if it is the case it does not diminish the importance of the between-groups differences.

We previously reported data from the animals used in this study demonstrating that individuals with extreme right asymmetric frontal electrical activity display an increase in fear-related behaviors when compared to a group of animals with extreme left frontal activity. Extreme right frontal animals also had increased basal plasma cortisol concentrations when assessed at 14 months of age, and these difference persisted up to 40 months of age (Kalin et al 1998a). We suggested that in primates, this constellation of behavioral and physiological characteristics constitutes an endophenotype for fearful temperament. The current findings add to this characterization by demonstrating that, compared to extreme left frontal monkeys, extreme right frontal monkeys have increased CSF CRH concentrations. Importantly, these differences were apparent when the monkeys were infants, as early as 4 months of age, and continued into late adolescence, 52 months of age.

The increased baseline plasma cortisol concentrations occurring in right frontal monkeys indicates increased basal activity of the pituitary–adrenal system; whether this is due to increase activity of PVN CRH neurons is unclear. The increased CSF CRH in these animals suggests increased availability of this neuropeptide in regions such as cortex, amygdala, and/or brain stem. Because of the established actions of CRH on increasing fearful behavior in rodents and primates (Kalin et al 1983, 1989; Sutton et al 1982; Takahashi et al 1989), it is tempting to speculate that increased activity in extrahypothalamic CRH neurons, as indexed by increased CSF CRH, could underlie the increase in fearful behaviors. No direct evidence exists that mechanistically links increased CRH activity to asymmetric right frontal electrical activity; however, the anxiolytic agent, diazepam, shifts monkeys' asymmetric frontal electrical activity from right to left frontal regions (Davidson et al 1992), and in rats benzodiazepines decrease regional brain concentrations of CRH (Owens et al

1991). It is possible that the effect of benzodiazepines on shifting frontal electrical activity from right to left could be mediated by the benzodiazepine-induced reduction in CRH.

The amygdala is a site where increased CRH activity could be mediating some of the behavioral and physiological characteristics associated with the fearful temperament endophenotype. The amygdala has a high density of CRH-containing neurons, which are primarily located in the central nucleus of the amygdala CeA (Swanson et al 1983) and the CeA projects to sites that are important in mediating stress and fear-related responses (Davis 1997; LeDoux 1987). For example, projections to the PVN promote stress-induced activation of the HPA system, and projections to the periaqueductal gray are important in mediating freezing behavior. Furthermore, in rodents certain stressors elevate CeA CRH mRNA concentrations (Hsu et al 1998; Kalin et al 1994) and increase extracellular CeA CRH concentrations (Merali et al 1998; Pich et al 1995). The intraventricular administration of CRH antagonists block stress-induced behavioral and physiological effects (Heinrichs et al 1992; Kalin et al 1998), and data from site-specific administration studies suggest that the CeA is one site at which endogenous CRH mediates its behavioral effects (Swiergiel et al 1993). The basolateral region of the amygdala is bidirectionally linked to cortical areas and has a high density of CRH receptors. Thus, efferents from the basolateral region projecting to prefrontal cortex could be important in mediating the pattern of asymmetric right frontal electrical activity associated with fearful temperament.

Recent data suggests that early experience may play a role in the development of individual differences in the fearful endophenotype. Research has demonstrated that brief handling of rat pups results in an attenuated stress-induced HPA response when these pups are tested as adults (Heim et al 1997; Levine 1957; Plotsky et al 1993). These animals also have decreased PVN and CeA CRH mRNA concentrations. In contrast, rat pups that are exposed to repeated maternal separations during the first 2 weeks of life have elevated levels of PVN and CeA CRH mRNA (Heim et al 1997; Plotsky and Meaney 1993). Maternal rearing style may be an important variable, as offspring of mothers that engage in low levels of grooming and licking have, as adults, an enhanced HPA response and increased PVN CRH mRNA levels (Liu et al 1997). Particularly meaningful to the present study are findings from bonnet macaques demonstrating that infants reared by mothers who are confronted with unpredictable foraging conditions have persistent elevations in CSF CRH concentrations (Coplan et al 1996).

In summary, the current findings demonstrate that in nonhuman primates CSF CRH levels are trait-like and are associated with electrophysiological, hormonal, and behavioral characteristics of fearful temperament. Along with other preclinical and clinical studies, these findings support the possibility that extrahypothalamic brain CRH systems play a role in mediating fearful temperament in humans.

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References

Altemus M, Pigott T, Kalogeras KT, Demitrack M, Dubbert B, Murphy DL, et al (1992): Abnormalities in the regulation of vasopressin and corticotropin releasing factor secretion in obsessive-compulsive disorder. *Arch Gen Psychiatry* 49:9–20.

Banki CM, Bissette G, Arato M, O'Connor L, Nemeroff CB (1987): CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *Am J Psychiatry* 144:873–877.

Banki CM, Karmacsi L, Bissette G, Nemeroff CB (1992): CSF corticotropin-releasing hormone and somatostatin in major depression: Response to antidepressant treatment and relapse. *Eur Neuropsychopharmacol* 2:107–113.

Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, et al (1997): Elevated CSF corticotropin-releasing factor concentrations in post-traumatic stress disorder. *Am J Psychiatry* 154:624–629.

Butler PD, Weiss JM, Stout JC, Nemeroff CB (1990): Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into the locus coeruleus. *J Neurosci* 10:176–183.

Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, et al (1996): Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for that pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci U S A* 93:1619–1623.

Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ (1997): Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: Effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J Pharmacol Exp Ther* 281:163–172.

Davidson RJ, Kalin NH, Shelton SE (1992): Lateralized effects of diazepam on frontal brain electrical asymmetries in rhesus monkeys. *Biol Psychiatry* 32:438–451.

Davidson RJ, Kalin NH, Shelton SE (1993): Lateralized response to diazepam predicts temperamental style in rhesus monkeys. *Behav Neurosci* 107:1106–1110.

Davidson RJ (1995): Cerebral asymmetry, emotion and affective style. In: Davidson RJ, Hugdahl K, editors. *Brain Asymmetry*. Cambridge, MA: MIT press, 361–387.

Davidson RJ (1998): Anterior electrophysiological asymmetries, emotion and depression: Conceptual and methodological conundrums. *Psychophysiology* 35:607–614.

Davis M (1997): Neurobiology of fear responses: The role of the amygdala. *J Neuropsychiatry Clin Neurosci* 9:382–402.

De Souza EB (1995): Corticotropin-releasing factor receptors: Physiology, pharmacology, biochemistry and role in central nervous system and immune disorders. *Psychoneuroendocrinology* 20:789–819.

De Souza EB, Insel TR, Perrin MH, River J, Vale WW, Kuhar MJ (1985): Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: An autoradiographic study. *J Neurosci* 5:3189–3203.

Geracioti TD, Loosen PT, Orth DN (1997): Low cerebrospinal fluid corticotropin-releasing hormone concentrations in eucortisolemic depression. *Biol Psychiatry* 42:165–174.

Heim C, Owens MJ, Plotsky PM, Nemeroff CB (1997): The role of early adverse life events in the etiology of depression and post-traumatic stress disorder. Focus on corticotropin-releasing factor. *Ann N Y Acad Sci* 821:194–207.

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF (1992): Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. *Brain Res* 581:190–197.

Hsu DT, Chen F-L, Takahashi LK, Kalin NH (1998): Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus: An in situ hybridization analysis. *Brain Res* 788:305–310.

Kagan J, Reznick JS, Snidman N (1988): Biological bases of childhood shyness. *Science* 240:167–171.

Kalin NH, Larson C, Shelton SE, Davidson RJ (1998a): Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. *Behav Neurosci* 112:286–292.

Kalin NH, Shelton SE (1989): Defensive behaviors in infant rhesus monkeys: Environmental cues and neurochemical regulation. *Science* 243:1718–1721.

Kalin NH, Shelton SE, Barksdale CM (1989): Behavioral and physiologic effects of CRH administered to infant primates undergoing maternal separation. *Neuropsychopharmacology* 2:97–104.

Kalin NH, Shelton SE, Barksdale CM, Brownfield MS (1987): A diurnal rhythm in cerebrospinal fluid corticotropin-releasing hormone different from the rhythm of pituitary–adrenal activity. *Brain Res* 426:385–391.

Kalin NH, Shelton SE, Rickman M, Davidson RJ (1998b): Individual differences in freezing and cortisol in infant and mother rhesus monkeys. *Behav Neurosci* 122:251–254.

Kalin NH, Shelton SE, Kraemer GW, McKinney WT (1983): Associated endocrine, physiological and behavioral changes in rhesus monkeys after intravenous corticotropin-releasing factor administration. *Peptides* 4:211–215.

Kalin NH, Sherman JE, Takahashi LK (1988): Antagonism of endogenous CRH systems attenuates stress-induced freezing behavior in rats. *Brain Res* 457:130–135.

Kalin NH, Takahashi LK, Chen F-L (1994): Restraint stress increases corticotropin-releasing hormone mRNA content in the amygdala and paraventricular nucleus. *Brain Res* 656: 182–186.

LeDoux JE (1987): Emotion. In: Plum F, editor. *Handbook of Physiology: Sec. 1. The Nervous System, vol. 5, Higher Functions of the Brain, Part 1*. Bethesda, MD: American Physiology Society, 419–459.

Levine S (1957): Infantile experience and resistance to physiological stress. *Science* 126:405–406.

Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al (1997): Maternal care, hippocampal glucocorticoid receptors, and hypothalamic–pituitary–adrenal responses to stress. *Science* 277:1659–1662.

Lyons DM, Wang OJ, Lindley SE, Levine S, Kalin NH, Schatzberg AF (1999): Separation induced changes in squirrel monkey hypothalamic–pituitary–adrenal physiology resemble aspects of hypercortisolism in humans. *Psychoneuroendocrinology* 24:131–142.

Merali Z, McIntosh J, Kent P, Michaud D, Anisman H (1998): Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. *J Neurosci* 18:4758–4766.

Nemeroff CB, Bissette G, Akil H, Fink M (1991): Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy. Corticotrophin-releasing factor, beta-endorphin and somatostatin. *Br J Psychiatry* 158:59–63.

Nemeroff CB, Owens MJ, Bissette G, Andorn AC, Stanley M (1988): Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch Gen Psychiatry* 45:577–579.

Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson L, Eklund K, et al (1984): Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* 226:1342–1344.

Owens MJ, Vargas MA, Knight DL, Nemeroff CB (1991): The effects of alprazolam on corticotropin-releasing factor neurons in the rat brain: Acute time course, chronic treatment and abrupt withdrawal. *J Pharmacol Exp Ther* 258:349–356.

Pich EM, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, et al (1995): Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci* 15: 5439–5447.

Plotsky PM, Meaney MJ (1993): Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA median eminence CRF content and stress-induced release in adult rats. *Brain Res Mol Brain Res* 18:195–200.

Plotsky P, Vale W (1984): Hemorrhage-induced secretion of corticotropin-releasing factor-like immunoreactivity into the rat hypophysial portal circulation and its inhibition by glucocorticoids. *Endocrinology* 114:164–169.

Raadsheer FC, Hoogendijk WJG, Stam FC, Tilders FJH, Swaab DF (1994): Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* 60:436–444.

Raadsheer FC, van Heerikhuize JJ, Lucassen PJ, Hoogendijk

WJG, Tilders FJH, Swaab DF (1995): Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. *Am J Psychiatry* 152:1372–1376.

Sutton RE, Koob GF, Le Moal M, Rivier J, Vale WW (1982): Corticotropin releasing factor produces behavioural activation in rats. *Nature (Lond)* 297:331–333.

Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983): Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: An immunohistochemical study. *Neuroendocrinology* 36:165–186.

Swiergiel AH, Takahashi LK, Kalin NH (1993): Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala in the rat. *Brain Res* 623:229–234.

Swiergiel AH, Takahashi LK, Rubin WW, Kalin NH (1992): Antagonism of corticotropin-releasing factor receptors in the locus coeruleus attenuates shock-induced freezing in rats. *Brain Res* 587:263–268.

Takahashi LK, Kalin NH, Vanden Burgt JA, Sherman JE (1989): Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. *Behav Neurosci* 103:648–654.

Tomarken AJ, Davidson RJ, Wheeler RE, Doss RC (1992): Individual differences in anterior brain asymmetry and fundamental dimensions of emotion. *J Pers Soc Psychol* 62:676–687.

Vale W, Spiess J, Rivier C, Rivier J (1981): Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213: 1394–1397.