

## Persistent central nervous system effects of an adverse early environment: clinical and preclinical studies

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### Abstract

In the search for the underlying biological causes of psychiatric disorders, primary roles for both genetics and environment have been clearly established. A family history of mood or anxiety disorders, representing the genetic component, clearly increases the risk for developing these illnesses in adulthood. The pivotal role of early environmental influences in the pathogenesis of these disorders is also supported by an abundance of both clinical and preclinical data. This review will highlight some of the preclinical and clinical literature that suggests early adverse experience may sensitize corticotropin-releasing factor (CRF) circuitry. The neurobiology of depression highlighting the pathophysiological role of CRF is reviewed. Next, some of the preclinical models of early life stress are discussed; along with a review of the relevant clinical literature that suggests that the functional dysregulation of CRF circuitry in response to early life trauma may contribute to adulthood depression. The discussion will be framed in regards to a stress-diathesis model in which early adverse events result in a sensitized stress axis that predisposes individuals to develop mood disorders.

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### 1. Introduction

In the search for the underlying biological causes of psychiatric disorders, primary roles for both genetics and environment have been clearly established. A family history of mood or anxiety disorders, representing the genetic component, clearly increases the risk for developing these illnesses in adulthood. The pivotal role of early environmental influences in the pathogenesis of these disorders is also supported by an abundance of both clinical and preclinical data. The importance of the mother–child bond in humans was discussed in detail by Freud over a 100 years ago. However, the critical influence between early environment and behavior was first clearly demonstrated by Harlow in his seminal studies of maternally deprived rhesus monkeys [1]. Rhesus monkeys who spent the first 6 months of their life in partial isolation (i.e. raised with peers in the absence of the mother) exhibited a number of exaggerated oral behaviors, stereotypic movements, heightened fear and

aggression, and a reduced ability to deal with daily stressors [2]. Early work in primate models was soon replicated in other species in the late 1950s and 1960s [3].

Epidemiological studies have revealed a disturbingly high rate of child maltreatment in the United States. The National Center of Child Abuse and Neglect reports over 1.5 million confirmed cases of child maltreatment each year; more than 50% of these cases represent neglect, and about 700,000 cases are compromised of sexual, emotional, or physical abuse [4]. Combining these data with the observation that early untoward life events constitute a major risk factor for the development of certain psychiatric disorders, the need for a better understanding of the neurobiological consequences of early life stress is essential. Maltreated children have an increased rate of major depression, post-traumatic stress disorder (PTSD), attention deficit/hyperactivity disorder, and other behavioral disorders [5–9].

A survey of approximately 2000 adult women from four internal medicine practices demonstrated that a history of childhood sexual or physical abuse, but not adulthood rape or physical assault, greatly increased the frequency of symptoms of depression and anxiety, including suicidality, relative to women without a history of childhood abuse [10]. Recent

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traumatic or stressful events are also known to influence the course and severity of mood disorders in adulthood [11–14]. The concatenation of the myriad of clinical findings implicating the roles of genetic, childhood, and recent stressors in the etiology of depression has led several research groups, including our own, to propose a stress-diathesis model in which early untoward life events result in a permanent sensitization of neuroadaptive mechanisms to stressful life events; in turn this sensitized stress axis becomes overactive in response to subsequent, even mild, stressful events. As a consequence, when such individuals are exposed to stressful life events later in life, they likely hyperrespond, leading to a maladaptive cascade of events. The limbic lobe of MacLean's triune brain appears particularly vulnerable to these effects. Although beyond the scope of this review, a wealth of research has demonstrated that prenatal stressors can also have long-term consequences on both behavioral and endocrine parameters [15,16].

In this short review, we will first discuss the neurobiology of depression highlighting the pathophysiological role of corticotropin-releasing factor (CRF). We next highlight some of the preclinical models of early life stress, and conclude with a review of the relevant clinical literature that suggests that the functional dysregulation of CRF circuitry in response to early life trauma may contribute to adulthood depression.

## 2. CRF and its role in the neurobiology of depression

In the search for the underlying etiology of psychiatric disorders, countless neurotransmitter and hormone imbalance hypotheses have been promulgated to explain the biology of psychiatric illness. More than 50 years ago, it was reported that a large subset of depressed patients hypersecrete cortisol. In fact, this remains one of the most venerable findings in all of biological psychiatry. With the subsequent discovery of CRF, a new level of understanding of the hypothalamic–pituitary–adrenal (HPA) axis and its role in depression became possible. As will be discussed in more detail below, CRF circuits play a vital role in the modulation of affect, which is best exemplified by the finding that intracerebroventricular injection of CRF reproduces many of the hallmark symptoms of depression and anxiety in laboratory animals including decreased libido, reduced appetite and weight loss, sleep disturbances, and neophobia [17,18].

CRF, also known as corticotropin-releasing hormone (CRH), is a 41 amino acid containing peptide synthesized in parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus. CRF-containing cells in the PVN receive input from a variety of brain nuclei including the amygdala, bed nucleus of the stria terminalis, and other brainstem nuclei [19]. CRF-containing neurons in the PVN project to nerve terminals in the median eminence [20], where CRF is released into the hypophyseal–portal system

where it promotes the synthesis of pro-opiomelanocortin (POMC) and the release of adrenocorticotropin hormone (ACTH),  $\beta$ -endorphin, and others. ACTH released from the anterior pituitary in turn stimulates the production and release of cortisol, the primary glucocorticoid in humans, from the adrenal cortex. In addition to this hypothalamic CRF projection, several extrahypothalamic CRF systems have now been described in detail, including those in limbic and brainstem areas.

## 3. The biology of CRF

With the discovery of CRF [21], synthetic CRF soon became available which has allowed a comprehensive assessment of the HPA axis to proceed. It is now well established that CRF coordinates the endocrine, immune, autonomic, and behavioral responses of mammals to stress. CRF exerts its effects through two CRF receptor subtypes, CRF<sub>1</sub> and CRF<sub>2</sub>, which have distinct localization and receptor pharmacology [22–27]. In addition, a putative CRF<sub>3</sub> receptor has recently been identified in catfish [28]. The CRF<sub>1</sub> receptor is predominantly expressed in the pituitary, cerebellum, and neocortex in the rat [29], whereas CRF<sub>2</sub> receptors are more prevalent in subcortical regions, such as the ventromedial hypothalamus, the lateral septum, and the dorsal raphe nucleus. A growing body of evidence from animal studies suggests that the CRF<sub>1</sub> receptors may specifically mediate some of the anxiogenic-like behaviors observed after administration of CRF [30–32]. In addition to CRF, additional members of the CRF family include urocortin, and urocortin II and III (also known as stresscopin and human stress related peptide) [33]. With the discovery of new ligands and a putative third receptor in the CRF family, much of the pharmacology and functional interactions between these ligands and receptors remains to be discovered [34].

## 4. HPA axis abnormalities in depression

The occurrence of depression and other psychiatric symptoms in both Cushing's and Addison's disease served as an impetus for researchers to scrutinize HPA axis abnormalities in depression and other psychiatric disorders. The earliest studies in this field demonstrated elevated plasma cortisol concentrations in depressed patients [35,36]. Other markers of hypercortisolemia that have been reliably demonstrated in depressed patients include elevated 24-h urinary-free cortisol concentrations and increased levels of cortisol metabolites in urine [37].

Several stimulation tests have also been developed to assess the functional integrity of the HPA axis. One commonly used test is the dexamethasone suppression test (DST). In this test, 1 mg of dexamethasone is administered at 11 p.m., blood is then obtained at 8 a.m., 4 p.m. and 11 p.m. the following day and cortisol levels are measured.

Dexamethasone is a synthetic glucocorticoid similar to cortisol, and suppresses ACTH secretion, and subsequently cortisol release, in healthy volunteers. Nonsuppression of plasma glucocorticoid levels following the administration of dexamethasone is common in depression. The rate of cortisol nonsuppression after dexamethasone administration generally correlates with the severity of depression [38]; in fact nearly all patients with major depression with psychotic features exhibit DST nonsuppression [39,40].

Another method used to assess HPA axis activity is the CRF stimulation test. In this paradigm, synthetic ovine or human CRF is administered intravenously (usually a 1 µg/kg dose), and the ensuing ACTH and cortisol response is measured at 30-min intervals over a 2–3 h period [41]. Numerous studies have now demonstrated a blunted ACTH and β-endorphin response to exogenously administered ovine CRF (oCRF) or human CRF (hCRF) in depressed patients when compared to nondepressed subjects, though the cortisol response in depressed patients and nondepressed control subjects did not consistently differ [42–46]. The attenuated ACTH response to CRF is presumably due to either chronic hypersecretion of CRF from nerve terminals in the median eminence, which results in down-regulation of CRF receptors in the anterior pituitary, and/or the chronic hypercortisolemia providing negative feedback on ACTH secretion from the pituitary. This receptor down-regulation results in a reduced responsiveness of the anterior pituitary to CRF, as has been demonstrated in laboratory animals [47–51].

A combined dexamethasone/CRF test has also been developed. In this test, 1.5 mg of dexamethasone is administered orally at night (2300 h), and subjects receive an intravenous bolus of 100 µg of human CRF at 1500 h the following day. Patients with HPA axis dysfunction, which is frequently encountered in depression, display an increased release of ACTH and cortisol relative to controls, i.e. they escape dexamethasone suppression [52]. These abnormalities essentially normalize following remission of depression, and this precedes full clinical remission [52,53]. The combined DEX/CRF test appears to have much higher sensitivity for detecting subtle alterations in HPA axis function, and approximately 80% of patients with major depression exhibit an abnormal response to the DEX/CRF test. In contrast, only approximately 44% of patients with major depression demonstrate an abnormal DST response [52,53].

The studies thus far discussed focused primarily on dysregulation of the HPA axis, but as noted earlier, CRF controls not only the neuroendocrine, but also the autonomic, immune, and behavioral responses to stress in mammals. Results from both clinical studies, and a rich body of literature conducted primarily in rodents and lower primates, have indicated the importance of CRF independent of its classical role in regulating the HPA axis. In rodents, primates, and humans, CRF and its receptors have been heterogeneously localized in a variety of regions including the amygdala, thalamus, hippocampus, and prefrontal cortex, among others [54–57]. These brain regions are impor-

tant in regulating many aspects of the mammalian stress response, and in regulating affect. The presence of CRF receptors in both the dorsal raphe (DR) and locus coeruleus (LC), the major serotonergic and noradrenergic-containing regions in the CNS, respectively, also deserves comment. Because most available antidepressants, including the tricyclics and selective serotonin reuptake inhibitors (SSRI), are believed to work via modulation of noradrenergic and/or serotonergic systems, the neuroanatomical proximity of CRF and monoaminergic systems suggests a possible site of interaction between CRF systems and antidepressants. It may even be that the long latency of antidepressant therapeutic action is due to their effects on CRF neurons.

Involvement of extrahypothalamic CRF systems in the pathophysiology of depression is suggested by numerous studies showing elevated CRF concentrations in the cerebrospinal fluid (CSF) of patients suffering from depression [58–62], though discrepant results have appeared [63]. Elevated CSF CRF concentrations have also been detected in depressed suicide victims [58]. A reduction in the density of CRF receptors in the frontal cortex has also been reported in the frontal cortex of suicide victims [64], and we have recently confirmed these findings in a second study. Presumably, CRF hypersecretion results in a down-regulation of CRF receptors in the frontal cortex. Two recent studies have also revealed increased CSF CRF levels in patients with both major depression and/or PTSD using serial lumbar CSF sampling techniques; the advantage of these studies is that it removes the confound associated with the initial lumbar puncture which itself is highly stressful [65,66]. These studies further support the hypothesis of elevated CRF concentrations in patients with depression and PTSD.

## 5. Animal models of early life stress

Despite the large number of abnormalities in the HPA axis and CRF systems discussed above, these studies do not necessarily indicate the underlying cause of these disturbances. However, recent focus has been placed on the role of social behavior and early life stressors. It is now clear that early experience influences the long-term development of behavioral, neuroendocrine, and cognitive systems in a number of animal species (including humans), and highlights the importance of a stable environment during the formative years. Numerous paradigms scrutinizing prenatal stress, neonatal maternal deprivation, and isolation rearing have been investigated in order to study the effects of adverse early experience on the development of the CNS.

## 6. Rodent models of early life stress

Several rodent models have been developed to further explore the effects of early life stressors, usually centered on

a separation of newborn rat pups from their dams. The specific details used in these paradigms vary greatly between research groups, ranging from a single 24-h deprivation to repeated, but shorter, maternal deprivation episodes lasting 12, 8, 6, or 3 h starting between postnatal days (PNDs) 1–2 and extending through PNDs 14–21 [67]. Additionally, some groups further separate the pups from one another during their separation period, whereas other researchers keep the pups together during the period of separation. As this topic is discussed elsewhere in this issue, we will only highlight some of the key points here.

In one implementation of the maternal separation paradigm employed by Plotsky and Meaney [68], on PND 2, all pups are removed from their home cage, randomized, and culled to 8–10 pups per group, usually consisting of 10 male pups per dam. Each litter is then exposed to one of three rearing conditions from PNDs 2 to 14: (a) animal facility rearing (AFR), which consists of home cage bedding material changes and brief handling twice weekly for routine cage changes beginning on PND 5 with no other handling or separation; (b) handled (HMS15) animals, which are removed from their home cage daily for 15 min periods; and (c) maternal separation (HMS180) in which pups are removed from their home cage for 180 min daily. In some experiments, an additional nonhandled group is included; in this condition, the mother and pups are left completely undisturbed for the first 14 days after birth, this also means there are no cage changes during this period. Animals are then weaned on PNDs 21–23 and group housed (two to three per cage) until adulthood.

As adults, maternally deprived rats (HMS180) exhibit a markedly elevated HPA axis response to stressors when compared to nondeprived littermates. In a defensive withdrawal test, adult rats who had been separated for 3 h from their mothers during PNDs 2–14 showed an increased latency to exit from the “safe” compartment, and spend a greater total amount of time in this area relative to non-separated litters [67]. Furthermore, maternally deprived rats spent less time in the open arms of an elevated plus maze relative to nondeprived animals, a validated model of anxiety. Interestingly, animals that were exposed to brief amounts of handling (AFR and HMS15 rats) demonstrated a reduced stress response relative not only to HMS180 animals, but also to nonhandled animals. Indeed, taken together, the evidence indicates that early life brief handling actually decreases the stress response in adults. These behavioral observations are consistent with the hypothesis that the postnatal environment profoundly influences the developing CNS.

Although basal plasma ACTH and corticosterone levels did not differ between rearing groups, maternally deprived rats exhibit exaggerated ACTH and corticosterone responses to psychological stressors. The response to an airpuff startle is greatly enhanced in maternally separated rats [69]. It appears that prolonged maternal separation results in an exaggerated HPA axis response to psychological stressors

(e.g. novel environment, airpuff startle, restraint). These results implicate alterations in the corticolimbic pathways coordinating the processing of higher-order sensory stimuli and their transfer to the hypothalamic PVN.

## 7. Animal models of early life stress and changes in CRF

Of considerable interest to this present review, the alterations in HPA axis function seen in maternally separated animals are associated with changes in the expression patterns of CRF mRNA. Hypothalamic CRF mRNA, specifically in the PVN, is markedly elevated in maternally deprived rats [68]. HMS180 rats also exhibit elevated CRF and AVP concentrations in the hypophyseal–portal circulation relative to handled (HMS15) rats, consistent with the elevated CRF mRNA expression observed in the hypothalamus. In addition, hypothalamic CRF receptor binding is increased in maternally deprived rats relative to handled (HMS15) rats. This later finding suggests increased CRF<sub>1</sub> receptor mRNA expression in the PVN as a consequence of the maternal deprivation stress. The increased CRF content along with increased binding demonstrated in these animals would also suggest an increased responsiveness of these animals to the effects of CRF.

There is evidence that maternal deprivation induces changes not only in the HPA axis, but also in various extrahypothalamic sites involved in the coordination and integration of sensory information. CRF mRNA levels in the bed nucleus of the stria terminalis and central nucleus of the amygdala are elevated in maternally deprived rats and decreased in HMS15 handled rats relative to nonhandled controls [67]. These CRF pathways project directly to brainstem nuclei, including the LC, where CRF directly activates noradrenergic neurons [70]. Because the LC is involved in regulating the autonomic nervous system, these pathways may help enhance vigilance behavior and further drive the HPA axis. Changes in both norepinephrine and central benzodiazepine systems have also been demonstrated [71,72]. The increased CRF and the reduced central benzodiazepine and  $\alpha_2$ -adrenergic receptor binding demonstrated in these maternally deprived rats provide a general mechanism to explain the observed stress hyperresponsiveness seen in these animals.

The results from animal models of mood and anxiety disorders lend validity to a stress-diathesis model in which both genetic and environmental factors interact in order to regulate complex behaviors, such as those involved in responding to a stressful and changing environment. Although we have focused primarily on the neurobiological sequelae of prolonged periods of maternal deprivation, short periods of handling during the postnatal period may have neuroprotective or anxiolytic effects in certain instances. Relative to nonhandled animals, rodents handled during infancy spent more time exploring a novel environment when tested as adults [73], and showed reduced emotional

reactivity to handling [74]. A recent study examined differences in the amount of pup licking and grooming and arched-back nursing revealed that pups with mothers who showed the least licking and grooming behavior exhibited a phenotype similar to HMS180 animals; in contrast, pups of mothers who exhibited high licking and grooming behavior demonstrated a phenotype similar to AFR or handled animals. Thus, it is reasonable to conclude that extended maternal separation may induce alterations in the mother–pup interaction, analogous to maternal neglect in humans, resulting in the altered behavioral phenotype seen in these animals [75,76].

## 8. Primate studies of maternal separation

Experiments in primates have yielded similar results to those observed in rodents. Adult nonhuman primates maternally deprived during infancy demonstrate elevated HPA and behavioral responses to stressful stimuli [77]. Exposing mother–infant bonnet macaques to varying levels of foraging demand over a 12-week period induced severe behavioral disturbances in the infants. The study was comprised of three groups: a low foraging demand (LFD) condition, where the mothers can easily obtain food; a high foraging demand (HFD) condition, where mothers had to complete a daily task in order to obtain food; and a variable foraging demand (VFD) condition, where the accessibility of food was unpredictable. It is believed that the lack of predictability results in a diminished sense of security as to the availability of food, resulting in an increase in anxiety in the mother with a subsequent reduction in the quality of maternal care.

When allowed to grow into adulthood, VFD-reared animals demonstrate an anxious temperament and significantly elevated CSF CRF concentrations relative to LFD- or HFD-reared primates [78]. VFD-reared animals also appear to have alterations in noradrenergic and serotonergic functions as a result of early life stress [79,80]. Overall, these results are consistent with the rodent literature and further demonstrate that early social interactions can cause permanent changes in the stress axis.

## 9. Clinical studies

Considering both the clinical data and preclinical data presented above, a link between the dysregulation of CRF systems as a result of early untoward events seems compelling. However, despite the rich preclinical literature available and the epidemiological studies suggesting a link between childhood trauma and psychopathology (see Introduction), the available clinical data concerning the biological sequelae of early life stress in children are relatively limited. Several studies have demonstrated that children exposed to early life stress at different developmental stages

have a disturbance of the circadian rhythm of HPA axis function [81]. De Bellis et al. [82] reported a markedly blunted ACTH response to CRF in sexually abused girls, most of whom were suffering from dysthymia, relative to controls. However, another study showed enhanced ACTH and normal cortisol responses in abused children with current depression when compared to nonabused depressed children and controls [83]. Clearly, more studies are needed, but these results demonstrate that neuroendocrine dysfunction as a result of early life stress is highly variable. Presumably, the type of abuse, age at onset, duration of the stressful event, time since the event, and ongoing psychopathology all play a critical role in the specific endocrine disturbances measured in children.

Overall, the disturbances of the HPA axis that are commonly encountered in adult depression such as hypercortisolemia or a blunted ACTH response to CRF stimulation are rare in depressed children [84,85]; however, it has been suggested that these measures may not reflect central CRFergic tone and that these peripheral markers may develop over time following more prolonged depression [86].

Several studies have also scrutinized the long-term consequences of early life stress in adults. We have recently measured HPA axis responses to stress in adult women with or without concurrent major depression who were exposed to severe physical or sexual abuse as children. In order to measure HPA axis responsivity, we employed the Trier Social Stress Test (TSST), which is a laboratory paradigm that involves a simulated 10-min public speech and a mental arithmetic task. The TSST has been validated as a potent activator of the HPA axis in humans [87]. We demonstrated increased HPA axis responses, presumably due to hypersecretion of CRF, after exposure to the TSST in women (both depressed and nondepressed) who were exposed to severe physical and emotional trauma as children [88]. Adults who experienced parental loss demonstrated similar findings [89]. Using a multiple regression model, we demonstrated that a history of childhood abuse, the number of separate abuse events, the number of adulthood traumas, and the severity of depression predicted the peak ACTH response to the psychosocial stress. Interestingly, the strongest predictor for ACTH response was a term that coded for the interaction between childhood (past) and adult (present) trauma. This correlation fits well with our model in which early untoward life events sensitize the stress responsive circuitry, so that upon re-exposure to traumatic events later in life, there is a hyperactive (and maladaptive) response in which CRF is hypersecreted, ultimately leading to the signs and symptoms of a major depressive episode.

## 10. Conclusion

The extant evidence clearly suggests that the quality of early social interactions can have long-reaching effects that

extend well into adulthood. The animal literature has shown both beneficial and harmful effects as a result of the quality of early social interactions. Human studies also indicate that severe childhood stressors including loss of a parent, neglect, or child abuse alter the prevalence of mood and anxiety disorders as adults. One important theoretical difference must be mentioned; the preclinical studies discussed have focused on a lack of care or neglect, whereas most of the clinical studies have focused on children or adults who were the subject of physical or sexual abuse. Despite this, the preclinical and clinical findings are remarkably concordant.

Although other neurotransmitter systems are clearly affected by adverse early events (see Ref. [90] for more details), a central involvement of CRF is highly suggested by both clinical and preclinical findings. With the recent work specifically linking disturbances in CRF function as a direct result of adverse early experiences (as seen in both rat and primate studies), a stress-diathesis model is compelling. Thus, analogous to the well-established critical periods in the development of the visual system [91], severe stress during the critical childhood years can cause serious abnormalities in stress responsive neural circuitry and alter the “set point” of the HPA axis to later stressful events, thus rendering victims of abuse more susceptible to developing depression or anxiety-related disorders later in life. Our “limbic inheritance” may make us particularly vulnerable to the effects of early adverse events. MacLean [92] clearly acknowledged that our limbic brain may undergo programming during adolescence, which may regulate how it interacts with the neocortex. It is particularly relevant that most of the effects of maternal separation seem to modulate the function of the amygdala and the hippocampus, which are key components of the limbic cortex. Thus, although clearly an oversimplification, one could envision that the effects of adverse early experiences may cause an imbalance in the normal functioning of the triune brain, which under stressful situations may result in “limbic overdrive.”

With a better understanding of the neurobiological sequelae of trauma, comes an increased ability to diagnose, and ultimately treat, mood and anxiety disorders. Indeed, newly developed CRF<sub>1</sub> receptor antagonists may represent a novel treatment strategy for depression, and may be highly efficacious in patients who were abused as children, due to the evidence of CRF hypersecretion in these patients.

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