The Role of Corticotropin-Releasing Factor–Norepinephrine Systems in Mediating the Effects of Early Experience on the Development of Behavioral and Endocrine Responses to Stress

Darlene D. Francis, Christian Caldji, Frances Champagne, Paul M. Plotsky, and Michael J. Meaney

Naturally occurring variations in maternal care in early postnatal life are associated with the development of individual differences in behavioral and hypothalamic–pituitary–adrenal responses to stress in the rat. These effects appear to be mediated by the influence of maternal licking and grooming on the development of central corticotropin-releasing factor (CRF) systems, which regulate the expression of behavioral, endocrine, and autonomic responses to stress through activation of forebrain noradrenergic systems. These findings provide a neurobiologic basis for the observed relationship between early life events and health in adulthood. In more recent studies, we explored the behavioral transmission of individual differences in stress reactivity, and thus, vulnerability to stress-induced illness, across generations. Biol Psychiatry 1999;46:1153–1166 © *1999 Society of Biological Psychiatry*

Key Words: CRF, stress, maternal behavior

Introduction

Illness in adulthood commonly occurs as a function of an existing high level of environmental demand, or stress, and an underlying vulnerability. Vulnerability can emerge as a function of genetic inheritance, early family life or, as is usually the case, both. Indeed, there often appears to be a close correlation between the nature of the genetic and familial factors (see Scarr and McCarthy 1983) such that vulnerability is most realistically viewed as the endpoint of converging influences. Such patterns are readily apparent in families with a history of depression. Children of depressed parents commonly inherit not only a genetic predisposition for depression but often, the compromised parental care of a depressed parent (see Field 1998). The enhanced vulnerability for depression derives from both genetic and behavioral modes of transmission.

The quality of the early family environment can serve as a major source of vulnerability in later life. Individuals who are the victims of physically or sexually abusive families are at considerably greater risk for mental illness in adulthood (e.g., Bifulco et al 1991; Brown and Anderson 1993). Perhaps somewhat surprisingly, persistent emotional neglect or conditions of harsh, inconsistent discipline serve to increase the risk of depression and anxiety disorders to a level comparable to that observed in more obvious cases of abuse (Holmes and Robbins 1987, 1988). Indeed, low scores on parental bonding scales, reflecting cold and distant parent–child relationships, also significantly increase the risk of depression in later life (e.g., Canetti et al 1997; Parker 1981). Children need not be beaten to be compromised. The risk is not unique to mental health. Russak et al (1997), in a 35-year follow-up of populations in the Harvard Stress Mastery Study, found that by midlife those individuals who as undergraduate students rated their relationship with parents as cold and detached, had a 4-fold greater risk of chronic illness, including not only depression and alcoholism but also heart disease and type II diabetes.

Individual differences in parental care are related to the health of the offspring. We argue that this effect is, in part at least, mediated by parental influences on the development of neural systems, which underlie the expression of behavioral and endocrine responses to stress (Figure 1). Parental rearing that results in enhanced reactivity to stress serves to increase the risk for illness in later life. The cornerstone of this argument is the fact that increased levels of stress hormones, notably the glucocorticoids and catecholamines, can promote the development of multiple
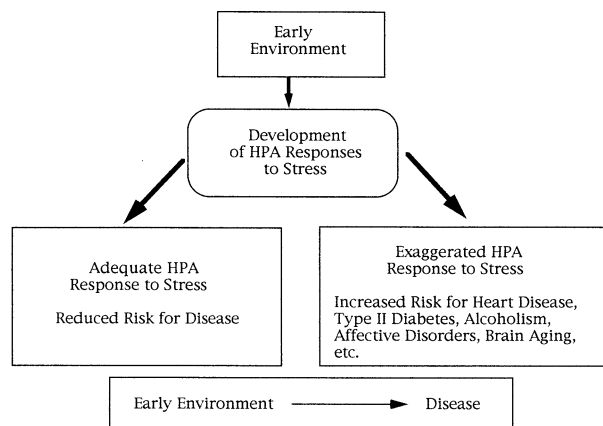
Figure 1. An outline of the the the developmental hypothesis for the role of individual differences in hypothalamic–pituitary–adrenal activity in mediating the relationship between environmental factors in early life and health in adulthood.

forms of chronic illness (see Chrousos and Gold 1992; McEwen 1998; Sapolsky 1994).

Stress and Health

Stress is a risk factor for several forms of illness. Interestingly, the effects of stress on health appear to be mediated by the activation of same systems that ensure survival. For example, the hypothalamic–pituitary–adrenal (HPA) response to stress is a basic adaptive mechanism in mammals that governs the metabolic and cardiovascular responses to the slings and arrows of everyday life, as well as to the more challenging conditions prevailing during chronic stress. During stress, the increased secretion of corticotropin-releasing factor (CRF) and arginine vasopressin (AVP) into the hypophysial–portal system of the anterior pituitary enhances the synthesis and release of proopiomelanocortin-derived peptides, adrenocorticotropin (ACTH) and β-endorphin (Plotsky 1991; Rivier and Plotsky 1986). Elevated ACTH levels, in turn, increase the synthesis and release of adrenal glucocorticoids. The highly catabolic glucocorticoids act in synergy with catecholamines to produce lipolysis, glycogenolysis, and protein catabolism, resulting in increased blood glucose levels (Baxter and Tyrrell 1987; Brindley and Rolland 1989; Munck et al 1984). These actions ensure the survival of the organism during stress, in part by increasing the availability of energy substrates. In addition, the delivery of energy substrates is enhanced by increased blood flow as a result of glucocorticoid- and catecholamine-induced increases in cardiovascular tone. Prolonged exposure to elevated stress hormone levels, however, can present a serious risk. Glucocorticoids and catecholamines promote the suppression of anabolic processes, muscle atrophy,

decreased sensitivity to insulin and a risk of steroid-induced diabetes, hypertension, hyperlipidemia, hypercholesterolemia, arterial disease, amenorrhea, impotency, and the impairment of growth and tissue repair, as well as immunosuppression (Baxter and Tyrrell 1987; Brindley and Rolland 1989; Munck et al 1984). In addition, there are essential changes in cognitive and emotional states during stress. Central CRF systems activate ascending serotonergic and noradrenergic pathways so that under conditions of threat, individuals become hypervigilant. Attention is focused on the source of the threat. Glucocorticoids and catecholamines enhance learning for information related to the stressor (see Cahill and McGaugh 1998), whereas glucocorticoids impair attention and learning in relation to events not directly associated with the stressor (see Lupien and Meaney 1998; McEwen et al in press for reviews). Although these responses are perfectly adaptive, continued activation of these same circuits results in impairments. Elevated central CRF activity is associated with symptoms of anxiety and depression, and these effects may well rest on the CRF-induced drive of serotonergic and noradrenergic systems (see Nemeroff 1996 and this volume). Exposure to chronically elevated glucocorticoid levels can result in hippocampal dysfunction and certain forms of cognitive impairments (Lupien and Meaney 1998; McEwen et al in press). Glucocorticoids also enhance the behavioral effects of central corticotropin-releasing hormone (CRH; Schulkin et al 1998). Glucocorticoids administered systemically potentiate the increases in fear-related startle observed with central CRH administration (Lee et al 1994). Specifically, glucocorticoids positively regulate CRH in the central nucleus of the amygdala (Schulkin 1999). Induction of CRH gene expression in the amygdala by glucocorticoids results in an enhanced state of anxiety in an animal (Schulkin et al 1998). Herein lies the dilemma: The same stress hormones that permit survival during stress can, ultimately, lead to disease.

These findings provide a basis for understanding how stress can influence health. Yet the influence of stress can only really be fully appreciated when we factor into the equation some appreciation of the individual's response to stress. Not all individuals fall sick under conditions of stress and questions concerning the basis for such individual differences are central to understanding the etiology of chronic disease. The hypothesis that guides our research focuses on the role of early life events in determining individual differences in vulnerability to stress-induced illness. Early life events permanently influence the development of central CRF systems which, in turn, mediate the expression of behavioral/emotional, autonomic, and endocrine responses to stress (Caldji et al in press; Coplan et al 1996; Kraemer et al 1989; Meaney et al 1996). In rodent and nonhuman primate populations, maternal deprivation

in infancy is associated with enhanced neural CRF gene expression and increased stress reactivity. In adulthood, these animals show greater activation of the HPA axis, sympatho-adrenomedullary systems, and central monoaminergic systems, and thus, greater vulnerability for stress-induced illness. Consequently, individual differences in endocrine and sympathetic responses to stress can serve as a source of vulnerability (or resistance) to pathology over the life span (Chrousos and Gold 1992; McEwen and Stellar 1993; Seckl and Meaney 1994). These findings provide a biologic basis for the link between early life trauma and illness in later life that has been so frequently defined in epidemiologic studies. The key assumption here is that early life events can alter the development of individual differences in stress reactivity.

Environmental Regulation of HPA and Behavioral Responses to Stress: Postnatal Handling Studies

Postnatal handling decreases behavioral and endocrine responses to stress in adulthood (Ader and Grota 1969; Bhatnagar et al 1995; Hess et al 1969; Levine 1957, 1962; Levine et al 1967; Meaney et al 1989; Viau et al 1993; Zarrow et al 1972). The central CRF systems are a critical target for these handling effects. This is not surprising considering the seminal role of these systems in mediating both behavioral and HPA responses to stress. Hypothalamic CRF neurons in the parvocellular region of the paraventricular nucleus of the hypothalamus (PVNh) regulate the release of pituitary ACTH and adrenal glucocorticoids. CRF neurons in the central nucleus of the amygdala (CnAmy) project to the locus coeruleus and increase the firing rate of locus coeruleus neurons, resulting in increased noradrenaline release in the vast terminal fields of this ascending noradrenergic system (see Gray and Bingaman 1996; Lavicky and Dunn 1993; Valentino et al 1998; van der Kooy et al 1984). One of the principal noradrenergic targets here is actually the CRF neurons of the PVNh. Noradrenaline is the major known source of drive over CRF release from PVNh neurons during stress (Plotsky 1991; Plotsky et al 1989). The activation of the CRF neurons of the PVNh is associated with increased activity in the nucleus tractus solitarius (NTS), the dorsal medullary nucleus (DMN), as well as the locus coeruleus (LC). Indeed, lesions of these regions, including the locus coeruleus, result in a significant decrease in stress-induced increased in extracellular PVNh levels of noradrenaline and reduced plasma ACTH responses (see Pacak et al 1995). The amygdaloid CRF projection to the LC (Koegler-Muly et al 1993; Moga et al 1989; van Bockstaele et al 1996) is also critical for the expression of behavioral responses to stress. Microinjections of the CRF receptor

antagonist, α-helical CRF, into the LC attenuate fear-related behaviors (Butler et al 1990; Sweirgel et al 1993). Hence, the CRF neurons in the PVNh and the central nucleus of the amygdala serve as important mediators of both behavioral and endocrine responses to stress.

As adults, neonatally handled (H) rats show decreased fearfulness in the face of novelty and more modest HPA responses to stress. These effects are related to altered CRF gene expression. First, H animals show decreased CRF mRNA expression in the PVNh and the CnAmy (see Plotsky and Meaney submitted; Viau et al 1993) and decreased CRF content in the locus coeruleus (Plotsky and Meaney submitted). Second, H animals show decreased CRF receptor levels in the locus coeruleus compared with nonhandled (NH) rats (Plotsky and Meaney submitted). Together, these findings suggest that there would be increased CRF-induced activation of the locus coeruleus during stress in the NH animals. At least two recent findings are consistent with this idea. By comparison to H rats, acute restraint stress in NH animals produces 1) a greater increase in cFOSir in the locus coeruleus (Pearson et al 1997); and 2) larger increases in extracellular noradrenaline levels in the PVNh (Liu et al in press). We propose that postnatal handling can decrease the expression of behavioral responses to stress by altering the development of the CnAmy-locus coeruleus CRF system.

Postnatal handling also effects systems that regulate CRF activity. The expression of CRF in PVNh neurons is subject to inhibitory regulation via glucocorticoid negative feedback. H rats show increased negative feedback sensitivity to glucocorticoids (Meaney et al 1989; Viau et al 1993). This effect is, in turn, related to the increased glucocorticoid receptor expression in the hippocampus and frontal cortex (Meaney et al 1985, 1989; O'Donnell et al 1994; Sarrieau et al 1988; Viau et al 1993), regions which are known to mediate the inhibitory effects of glucocorticoids over CRF synthesis in PVNh neurons (de Kloet 1991; Jacobson and Sapolsky 1991). Interestingly, glucocorticoids also serve to dampen stress-induced noradrenergic responses in the PVNh (Pacak et al 1995).

H rats also show increased $GABA_A$ receptor levels in the NAergic cell body regions of the locus coeruleus and the nucleus tractus solitarius as well as increased central benzodiazepine (CBZ) receptor levels in the basolateral and central nucleus of the amygdala, the locus coeruleus, and the nucleus tractus solitarius. The GABA/BZ system serves to inhibit CRF synthesis in the central nucleus of the amygdala (Owens et al 1991) and to reduce noradrenergic responses to stress (see Gray 1987). Together, these effects serve to either dampen CRF activity or to decrease the effect of CRF at the level of the locus coeruleus and NTS. Considering the importance of the central CRF systems in the regulation of stress responses, we feel that

this model provides a reasonable working hypothesis for the mechanisms underlying the handling effect on endocrine and behavioral responses to stress (see Caldji et al in press).

Prolonged Periods of Maternal Separation on HPA and Behavioral Responses to Stress

The handling procedure involves separating the pup from the dam for a period of ~15 min and exposing the pup to a novel array of sensory stimuli. In the course of normal mother–pup interactions, the dam is regularly away from the nest, and the pups, for periods of 20 to 30 min (Jans and Woodside 1990; Rosenblatt 1994). Thus, the handling manipulation does not result in an abnormal period of separation or loss of maternal care. But what about longer periods of separation, where there is a clear loss of maternal care? We (Plotsky and Meaney 1993) studied adult animals that were separated from their mothers once per day for 180 min from days 2 to 14 of life. This manipulation was based on the observation of Calhoun (1962) that in semi-naturalistic conditions, subordinate females were often obligated to locate nests at some distance from food and water sources, resulting in periods of separation that extended as long as 2 to 3 hours.

The long-term effects of HPA responses to stress were qualitatively different depending on the duration of maternal separation. As adults, animals exposed to repeated periods of maternal separation showed significantly increased plasma ACTH and corticosterone responses to either restraint or novelty stress compared to 0 min separation controls (animals reared in the same manner as NH animals). Animals exposed to the 15 min period of separation (animals reared in the same manner as H animals) showed reduced plasma ACTH or corticosterone responses to stress. The longer periods of maternal separation also resulted in decreased glucocorticoid receptor binding in the hippocampus, hypothalamus, and frontal cortex, and impaired negative feedback sensitivity. The negative feedback effect was apparent using exogenous glucocorticoid treatment (corticosterone or dexamethasone) to suppress either basal or stress-induced HPA activity (Plotsky and Meaney submitted). Interestingly, the maternal separation animals showed evidence for an "early escape" from low-dose dexamethasone suppression of basal ACTH levels so commonly seen on hypercorticoid, depressed humans. In this study, basal ACTH levels in maternal separation animals were suppressed at 4 but not 8, hours following treatment. As expected on the basis of the feedback differences, adult animals exposed to maternal separation for 180 or 360 min/day as neonates exhibited a marked increase in hypothalamic CRF mRNA and

median eminence CRF peptide content compared to either H or NH pups.

In addition to the effects on HPA responses to stress, the maternal separation animals also differed in behavioral and emotional responses to stress (Caldji et al in press; Plotsky and Meaney submitted; also see Patchev et al 1997). In brief, the maternal separation rats were virtually the opposite of the H animals on the entire series of measures described previously, especially amygdaloid measures of CRF mRNA expression and levels of CRFir in the locus coeruleus. Perhaps the most impressive difference between the NH and maternal separation animals, lay in the effect on CRF mRNA expression in the CnAmy (Plotsky and Meaney submitted); CRF mRNA levels in the CnAmy were 2-fold higher in the maternal separation animals compared with even the NH animals. Levels of CRFir were significantly increased in the locus coeruleus (Plotsky and Meaney submitted) and Ladd et al (1996) reported that maternal separation in rats increased CRFir in the neighboring parabrachial nucleus, which, along with the locus coeruleus, appears to be a major target for CRF projections from the CnAmy (see Gray 1990). Likewise, CRF receptor levels in the locus coeruleus were increased in maternal separation animals (Plotsky and Meaney submitted). In addition to the locus coeruleus, Ladd et al (1996) found that maternal separation increased CRF receptor binding in the raphé nuclei. These findings suggest that early-experience-induced changes in CRF systems might regulate both noradrenergic and serotonergic responses to stress (also see Higley et al 1991). Indeed, we found that PVNh levels of noradrenaline during a 20 min period of restraint stress were elevated in maternal separation animals by comparison to both H and NH rats (Liu et al in press). These findings are consistent with those of Lavicky and Dunn (1993), who showed that intracerebroventricular CRF infusion increased extracellular noradrenaline levels. Predictably, the maternal separation animals were highly fearful in behavioral tests of novelty (see Caldji et al in press). These effects involved reduced exploration or feeding in a novel environment and an increased acoustic startle responsivity.

The increased fearfulness of the adult animals exposed to prolonged periods of maternal separation in infancy was also associated with effects on the GABA$_A$/CBZ receptor system (Caldji et al in press). GABA$_A$ receptor binding was significantly reduced in the central and basolateral nucleus of the amygdala, as well as the frontal cortex. CBZ receptor binding in maternal separation animals was reduced in the central and basolateral nucleus of the amygdala, as well as the locus coeruleus and the nucleus tractus solitarius. These effects were associated with decreased expression of the mRNA for the γ_2 subunit of the GABA$_A$ receptor, which encodes for the CBZ site.

Moreover, the adult, maternal separation animals also showed increased expression of mRNAs for the α_2 and α_3 subunits, and decreased expression of the α_1 subunit mRNA (Caldji et al in press). This profile is associated with decreased GABA binding (see Wilson 1996). These findings suggest that the composition of the $GABA_A$ receptor complex is influenced by early life events. Considering the role of the GABAergic system in the inhibition of CRF-induced activation of ascending noradrenergic systems, it seems likely that these effects contribute to the increased fearfulness observed in the maternal separation animals. The focus of our current research lies in the hypothesis that the dampened GABAergic tone in the maternal separation animals contributes to the enhanced CRF expression in the amygdala and increased stress-induced activation of the noradrenergic systems.

Maternal separation during early postnatal life in the rat enhanced the magnitude of behavioral and HPA responses to stress. These findings are certainly comparable to those reported in rhesus monkeys (see especially the studies of Higley et al 1991). In addition, Kraemer et al (1989) have shown that repeated periods of maternal separation in early life increase CSF measures of central noradrenaline and 5-HT responses to social stress in the rhesus monkey. In humans, child abuse is associated with altered levels of stress reactivity and an increased risk for depression (DeBellis et al 1994; Heim et al 1997). Interestingly, Plotsky and Meaney et al (in press) have found that chronic administration of paroxetine reverses the effects of maternal separation on HPA responsivity to acute stress in the rat. This finding is consistent with those showing that antidepressants can decrease central CRF mRNA expression (Brady et al 1992) and increase glucocorticoid negative-feedback regulation of HPA activity (e.g., Rowe et al 1997). The results of these studies further underscore the importance of CRF action on ascending noradrenergic neurons as a critical site for "programming" by early life events and for antidepressant drug action (see Nemeroff et al 1991; Valentino et al 1998). Thus, the maternal separation studies appear to provide the basis for a useful model of vulnerability to depression (also see Plotsky and Meaney in press). Although the exaggerated level of HPA reactivity and decreased glucocorticoid feedback sensitivity of the maternal separation animals may suggest some functional similarity to depression, it would, we think, be unrealistic to consider these animals as a model of depression (or any other form of psychiatric disorder). Our assumption is that these animals model a predisease state of enhanced vulnerability associated with early life adversity.

The decreased mother–pup contact resulting from long periods of maternal separation seems likely to be a critical variable in understanding how this procedure increases behavioral and HPA responses to stress. But does this imply that under normal conditions, maternal care actively contributes to the development of neural systems that mediate stress responses, or simply that the absence of the mother is so disruptive to pup physiology that it affects the development of these systems? If maternal care is relevant, then what are the relevant features of mother–pup interactions, and how do they influence neural development? And what of the handling effects—do these findings bear any relevance for understanding the normal development of behavioral and endocrine responses to stress in response to natural variations in maternal care?

What Are the Critical Features of These Environmental Manipulations?

The handling paradigm has been useful in allowing us to examine the remarkable plasticity that exists within neural systems that mediate responses to stress, and to understand how individual differences in vulnerability to stress-related disease might emerge in response to early life events (see Seckl and Meaney 1994). At the same time, the artificial and nonspecific nature of the handling paradigm is unsettling (see Alberts 1994; Daly 1973). Normal development in a rat pup occurs in the rather dark, tranquil confines of a burrow, where the major source of stimulation is that of the mother and littermates. There is little that resembles the disruption associated with human handling. However, several authors, including Barnett (1967) and Levine (1975) have argued for the role of mother–pup interactions in mediating the effects of handling. Handling, although a brief interlude in the routine of mother–pup interactions, provides for an increase in sensory stimulation. This, in turn, alters the behavior and physiology of the pup; which are, of course, very relevant stimuli for the mother. Handling the pups does alter the behavior of the mother towards the offspring (Bell et al 1971; Lee and Williams 1974, 1975).

Such changes are apparent in the temporal patterning of the nest bouts. Typically, a nesting bout begins when the mother approaches the litter, gathers the pups under her, and suckles; and terminates when the mother licks the pups and leaves the nest (Rosenblatt 1994). Overall, mothers of H pups spend the same amount of time with their litters as mothers of NH pups. However, mothers of H litters have shorter, but more frequent nest bouts (Jans et al unpublished) and spend significantly more time licking and grooming (LG) their pups (Lee and Williams 1974, 1975; Liu et al 1997). The question, then, is whether this altered pattern of maternal behavior serves as a critical stimulus for the environmental effects on the development of endocrine and behavioral responses to stress.

We examined this question by recording naturally

occurring variations in mother–pup interactions in normally reared animals. We (Liu et al 1997) found considerable variation in two forms of maternal behavior: licking and grooming of pups and arched-back nursing (ABN). Arched-back nursing, also referred to as "crouching," is characterized by a dam nursing her pups with her back conspicuously arched and legs splayed outward. While common, it is not the only posture from which dams nurse. A blanket posture represents a more relaxed version of the arched-back position where the mother is almost lying on the suckling pups. As you can imagine, it provides substantially less opportunity for movements such as nipple-switching. Dams also nurse from their sides and often will move from one posture to another over the course of a nursing bout. Licking and grooming of pups occurs most frequently while the mother nurses in the arched-back position, so as you might imagine, the frequency of the two behaviors are closely correlated ($r = +.91$) across animals.

We then split the animals into two groups based on these behaviors: animals high or low in licking and grooming and arched-back nursing (LG-ABN). Note, there were no differences between these groups in the overall amount of time in contact with pups (Caldji et al 1998). Differences in the frequency of licking and grooming or arched-back nursing do not occur simply as a function of time in contact with pups. Finally, the results of three independent studies have failed to reveal any relationship between the frequency of licking and grooming or arched-back nursing and either litter size or gender composition (all r results $< .10$). The latter is an important consideration, since Moore et al (e.g., 1997) reported that male pups are licked more frequently than females. However, this refers only to anogenital licking. Our studies measure both anogenital and body licking.

We then allowed the offspring to mature to adulthood and examined HPA responses to stress. The logic here is simple. If handling-induced differences in licking and grooming or arched-back nursing are relevant for the effects on HPA development, then the offspring of High LG-ABN mothers should resemble the H animals. This is exactly what was found (Liu et al 1997). As adults, the offspring of High LG-ABN mothers showed reduced plasma ACTH and corticosterone responses to restraint stress. These animals also showed significantly increased hippocampal glucocorticoid receptor mRNA expression, enhanced glucocorticoid negative feedback sensitivity, and decreased hypothalamic CRH mRNA levels. In each case, these effects mimic those observed in H versus NH animals. Moreover, the magnitude of the corticosterone response to acute stress was significantly correlated with the frequency of both maternal licking and grooming ($r = -.61$) and arched-back nursing ($r = -.64$) during the

first 10 days of life, as was the level of hippocampal glucocorticoid receptor mRNA and hypothalamic CRH mRNA expression (all r results $> .70$; Liu et al 1997). These studies suggest that the critical feature for the handling effect on HPA development involves an increase in maternal licking and grooming. The results of these studies suggest that the behavior of the mother towards her offspring can "program" neuroendocrine responses to stress in adulthood.

The offspring of the high and low LG-ABN mothers also differed in behavioral responses to novelty (Caldji et al 1998). As adults, the offspring of the low LG-ABN, like NH animals, showed increased startle responses, decreased open-field exploration, and longer latencies to eat food provided in a novel environment. These animals also showed increased CRF receptor levels in the locus coeruleus and decreased CBZ receptor levels in the basolateral and central nucleus of the amygdala, as well as in the locus coeruleus (Caldji et al 1998) and increased CRF mRNA expression in the CnAmy (Francis et al unpublished). Predictably, stress-induced increases in PVNh levels of norepinephrine were significantly higher in the offspring of the low LG-ABN offspring (Caldji et al 1999). These differences map perfectly onto the differences in H and NH animals, and provide further support for the idea that the effects of handling are mediated by changes in maternal behavior.

It may surprise the reader that rather subtle variations in maternal behavior have such profound impact on development. However, for a rat pup, the first weeks of life do not hold a great deal of stimulus diversity. Stability is the theme of the burrow, and the social environment in the first days of life is defined by the mother and littermates. The mother, then, serves as a primary link between the environment and the developing animal. It seems reasonable that variations in mother–pup interaction would serve to carry so much importance for development. This seems to be particularly true for tactile stimulation. Tactile stimulation derived from the mother serves to dampen HPA activity, protecting the animal against the catabolic effects of stress hormones (see Levine 1994), and to foster growth-hormone release in the young (Schanberg et al 1984). Obviously, these effects serve to promote growth and development. Such stimulation also seems to alter the development of stress reactivity in adulthood.

The Stability of Individual Differences in Maternal Behavior

The differences in maternal behavior in the high and low LG-ABN mothers are not unique to the first litter (Francis et al 1998). Across 12 dams, in one study, there was a correlation of $+.84$ between the licking and grooming of

the first and second litters. If one animal was excluded from the data set, the correlation soars to +.95. Clearly, a high degree of correspondence in the behavior of the mothers across their two litters. The differences in maternal behavior are stable. These findings are comparable to those of primate studies in which individual differences in maternal behavior remained consistent across infants (see Fairbanks 1996). The effect of these individual differences in maternal licking and grooming and arched-back nursing on the development of fearfulness in the presence of novelty was as profound for litter 2 as for litter 1.

The Transmission of Individual Differences in Maternal Care to the Offspring

The results of these studies suggest that individual differences in behavioral and neuroendocrine responses to stress are, in part, derived from naturally occurring variations in maternal care. Mothers that show increased arched-back nursing and pup licking and grooming beget offspring with more modest responses to stress. It is easy to see how such effects might serve as a possible mechanism by which selected traits might be transmitted from one generation to another. Before we make make this leap, it is important to show that the individual differences in maternal behavior show intergenerational transmission. To study this question, we simply mated the offspring of high versus low LG-ABN mothers; male and female offspring of high LG-ABN mothers and the male and female offspring of low LG-ABN mothers. The female offspring of high LG-ABN mothers showed significantly more licking and grooming and arched-back nursing than did the female offspring of low LG-ABN mothers (Francis et al 1998). Hence, the differences in maternal behavior are transmitted from one generation to the next.

The intergenerational transmission of parental behavior has also been reported in primates. In rhesus monkeys there is clear evidence for family lineages expressing child abuse (Maestripieri 1998, 1999). There is also evidence for transmission of individual differences in parental styles falling within the normal range. Fairbanks (1989) found that daughters reared by mothers that consistently spent a higher amount of time in physical contact with their offspring, became mothers who were similarly more attentive to their offspring. In rhesus monkeys, Berman (1990) found that the rate of rejecting the infant by mothers was correlated with the rejection rate of their mothers. In primates, such individual differences in maternal behavior may be revealed in juvenile, nulliparous females. Thus, we found that among juvenile female vervet monkeys, time spent in proximity to nonrelated infants was associated with the maternal behavior of their mothers (Meaney et al 1991). In all cases, these findings

were independent of the social rank of the mother. Equally impressive findings exist in humans, where Miller et al (1997) found that scores on parental bonding measures between a mother and her daughter were highly correlated with the same measures of bonding between the daughter and her child. These findings suggest a common process of intergenerational transmission of maternal behavior. The next question concerns the mode of transmission.

The Mode of Transmission: Genomic or Nongenomic?

High LG-ABN mothers are less fearful, and beget less fearful offspring. The question is whether these characteristics in the offspring occur as a function of genomic-based inheritance. Are the offspring of high LG-ABN mothers less fearful as a function of genetic transmission of traits which determine fearfulness? If this is the case, then the differences in maternal behavior may be simply an epiphenomenon, and not causally related to the development of individual differences in behavioral and neuroendocrine responses to stress. This is certainly a possibility since maternal behavior in the rat is related to fearfulness, and selective breeding studies suggest that variations in genotype can contribute to the development of fear-related behavior.

This question can be also expressed in terms of maternal behavior. The female offspring of high LG-ABN mothers are, as adults, high LG-ABN mothers. Likewise, the female offspring of low LG-ABN mothers become low LG-ABN mothers. The trait is stable. Thus, the individual differences in maternal behavior are transmitted from one generation to the next. The question is, how?

The issue is not one of inheritance, that much seems clear. The question concerns the mode of inheritance. We (Francis et al 1998) addressed this question using a variation of the study described previously in which we mated the male and female offspring of high and low LG-ABN mothers, highs with highs and lows with lows. The pups of the female offspring were then either handled or nonhandled during the first 2 weeks of life. Again, the offspring of the high LG-ABN mothers showed significantly more licking and grooming and arched-back nursing of pups than did the offspring of low LG-ABN mothers. Handling the pups of these mothers, as expected, increased maternal licking and grooming and arched-back nursing. Interestingly, handling affected the maternal behavior only of the female offspring of the low LG-ABN-derived mothers. Low LG-ABN mothers with handled pups showed significantly more licking and grooming and arched-back nursing of pups than did the low LG/ABN-derived mothers of nonhandled pups. This findings was predictable based on earlier studies (Lee and Williams

1974, 1975; Liu et al 1997). What was interesting is that handling had no affect on the maternal behavior of the high LG/ABN-derived females. Thus, handling pups did not increase maternal behavior in those animals, which naturally show high levels of licking and grooming and arched-back nursing.

We allowed the pups to grow to maturity. As adults, the animals showed the predictable differences in behavioral and HPA responses to stress. The handled offspring of low LG-ABN mothers did not differ from either the handled or nonhandled offspring of high LG-ABN mothers on measures of plasma corticosterone responses to stress or behavioral fearfulness under conditions of novelty. They were, after all, handled pups. Predictably, the nonhandled offspring of low LG-ABN mothers showed significantly increased HPA responses to stress and increased fearfulness in responses to novelty.

The critical part of the study concerns the maternal behavior of these animals. If the differences in maternal behavior are transmitted only through genetic inheritance, then the prediction is that the offspring of low LG-ABN mothers should also be low LG-ABN mothers, regardless of whether they were handled or not in early life. A behavioral mode of transmission would suggest that the maternal behavior of the handled offspring of low LG-ABN mothers should resemble that of high LG-ABN mothers, which is in character with the maternal behavior if not the genetic background of their mothers.

The answer is clear. The handled offspring of low LG/ABN-derived mothers did not differ from the offspring of high LG/ABN-derived mothers in their frequency of licking and grooming or arched-back nursing. The nonhandled offspring of low LG/ABN-derived mothers were, as we would expect, low LG-ABN mothers themselves. These findings provide evidence for a nongenomic mechanism of inheritance.

The same can be said for the effects on fearfulness. As adults, the offspring of the handled LG-ABN mothers, beneficiaries of high levels of maternal licking and grooming that they are, resembled the offspring of either H or NH, high LG-ABN mothers on measures of fearfulness. The offspring of nonhandled LG-ABN mothers, as we would expect, show greater fearfulness in novel surroundings. Hence, the handling experience was transmitted to the next generation via the alteration in maternal behavior.

Thus, it appears that individual differences in maternal behavior can be transmitted from one generation to the next through a behavioral mode of transmission. In support of this conclusion, we (Francis et al 1999) recently found that, as adults, the biologic offspring of low LG-ABN mothers cross-fostered onto high LG-ABN mothers are indistinguishable from the natural progeny of high LG-ABN mothers on behavioral measures of fearfulness.

Moreover, in the adult females, maternal behavior was typical of high LG-ABN mothers. Likewise, the adult offspring of high LG-ABN mothers reared by low LG-ABN dams resembled the normal offspring of low LG-ABN mothers.

These findings suggest that specific environmental events can alter the trajectory of development not only in the affected offspring, but into the next generation. In the early 1960s, Denenberg's group (1963, 1964) provided evidence for such nongenomic transmission. These researchers compared the offspring of handled–handled matings with those of nonhandled–nonhandled matings and found that, as adults, the offspring of the handled parents were significantly less fearful in response to novelty than were the offspring of nonhandled parents; thus providing evidence for a transgenerational effect. For reasons that we have never understood, despite being published in Nature, the results of this remarkable study have remained almost ignored. Our contribution to this story is to have identified maternal behavior as a potential mediator for such transgenerational effects.

Our findings are also consistent with the results of other laboratories using the cross-fostering technique as a test for maternal-mediation hypotheses. For example, the spontaneously hypertensive rat (SHR) is a strain bred for hypertension, which appears in adolescence. While the selective breeding suggests a genetic background, the expression of the hypertensive trait is also influenced by epigenetic factors (see McCarty and Gold 1996). SHR pups reared by wild-type, Wistar-Kyoto (WKY) mothers do exhibit hypertension to the extent of kin reared by SHR dams. When borderline hypertensive rats (BHR), a hybrid formed by SHR–WKY matings, are reared by WKY mothers, they do not express the spontaneous hypertensive phenotype.

In a recent collaboration with Anisman's group, we examined the potential effects of maternal behavior on the development of behavior and endocrine responses to stress in BALBc mice. The BALBc is normally a strain that is very fearful and shows elevated HPA responses to stress. However, BALBc mice cross-fostered to C57 mothers are significantly less fearful, with lower HPA responses to stress (Zaharia et al 1996). Importantly, C57 mothers lick and groom their pups about twice as frequently as BALBc mothers (Anisman et al 1998). If the genetic influence was paramount, then we would expect no such relationship between maternal behavior and phenotype. Comparable findings have emerged with rat strains. Typically, Fisher 344 rats are more responsive to novelty and have increased HPA responses to acute stress by comparison to Long-Evans rats. Moore and Lux (1998) reported that Long-Evans dams lick and groom their offspring significantly more often than do Fisher 344 mothers. These findings are

consistent with a behavioral transmission hypothesis. The nexus of this hypothesis is not to underestimate the importance of genetic-based inheritance, but to underscore the potential for traits to move from one generation to another via a behavioral mode of transmission that involves variations in maternal behavior.

Under normal circumstances, of course, BALBc mice are reared by BALBc mothers. The genetic and environmental factors conspire to produce an excessively fearful animal. This is usually the reality of nature and nurture. Genetic and environmental factors work in concert, and are often correlated (Scarr and McCartney 1983). Because parents provide both genes and environment for their biologic offspring, the offspring's environment is therefore, in part, correlated with their genes. The offspring's genes are correlated with those of the parents', and the parents' genes influence the environment they provide for the offspring. The reason why many epidemiologic studies based on linear regression models often find that the epigenetic factors, such as parental care, do not add predictive value above that of genetic inheritance is because of this correlation. The environment the parent provides commonly serves to enhance the genetic differences—they are redundant mechanisms. The knowledge of an animal's BALBc pedigree is sufficient to predict a high level of timidity in adulthood. Additional information on maternal care would statistically add little to the predictability—the two factors work in the same direction. But this is clearly different from concluding that the maternal care is not relevant, and the results of the cross-fostering studies attest to the importance of such epigenetic influences.

Redundancy is also a key feature of development in the brain. The brain has multiple routes to the same endpoint. What is the value of this process? It can provide for diversion. If the genetically determined trajectory is not adaptive for the animal, then the ability to move in the direction of the current environmental signal would be of adaptive value. This is why there is so much room for the influence of postnatal factors and why they can override earlier influences. Thus, we can completely alter the phenotype of the BALBc mouse if it is reared in the care of a C57 mother. Hence, environment can alter the genetically influenced trajectory. This, after all, is the adaptive value of plasticity.

Individual Differences in Maternal Behavior

Individual differences in parental care can influence the development of stress reactivity and thus, we think, vulnerability for chronic illness in later life. But what accounts for such variations in parental care?

Human clinical research suggests that the social, emo-

tional, and economic context are overriding determinants of the quality of the relationship between parent and child (Eisenberg 1990). Human parental care is disturbed under conditions of chronic stress. Conditions that most commonly characterize abusive and neglectful homes involve economic hardship, martial strife, and a lack of social and emotional support (see Eisenberg 1990). Such homes, in turn, breed neglectful parents. Perhaps the best predictor of child abuse and neglect is the parents' own history of childhood trauma. More subtle variations in parental care also show continuity across generations. Scores on the Parental Bonding Index, a measure of parent–child attachment, are highly correlated across generations of mothers and daughters (Miller et al 1997). In nonhuman primates, there is also strong evidence for the transmission of stable individual differences in maternal behavior (see Berman 1990; Fairbanks 1996).

Individual differences in behavioral and endocrine responses to stress in the rat are associated with variations in maternal care during infancy (Caldji et al 1998; Liu et al 1997). The adult offspring of mothers that exhibited a low frequency of pup licking and grooming and nursing in the arched-back or crouched posture showed increased HPA responses to stress and increased fearfulness by comparison to the offspring of high LG-ABN mothers. These individual differences in maternal care were very reliably transmitted from one generation to the next. As mothers, the daughters of high LG-ABN show significantly more licking and grooming and arched-back nursing than do the daughters of low LG-ABN mothers. Moreover, we have provided evidence for the idea that the inheritance of individual differences in maternal care can occur via a behavioral mode of transmission.

The stress responsivity of the offspring mirrors that of their mothers. Low LG-ABN mothers are more fearful than are high LG-ABN dams (Francis et al 1998), and likewise, their offspring are more fearful and timid than are those of high LG-ABN mothers. We believe that this is, in fact, a crucial point in understanding the basis for the transmission of individual differences in parental behavior. In the rat, maternal behavior emerges as a resolution of an interesting conflict (Rosenblatt 1994). Females rats, unless they are in late pregnancy or lactating, generally show an aversion towards pups. The novelty of the pups is a source of aversion for females, typical of the generally neophobic adult rat. Habituation to the novelty results in an altered set of responses towards pups. Thus, continuous exposure to the novel pups renders females more likely to exhibit maternal behavior. For responsive females the positive cues associated with pups emerge from tactile, gustatory, and auditory cues (see Stern 1997). Thus, pup stimuli can either be aversive, eliciting withdrawal, or positive, eliciting approach. The onset of maternal

Low levels of resources
High predation
Social, environmental impoverishment
etc...

**Environmental
Conditions**

Pessimism

Maternal Care

_____

Neural development

**Developmental
Signal F1**

↑ CRH gene
↓ GABAa/CBZ rec.
↓ GR rec.

? Oxytocin
Dopamine

**Developmental
Consequence
F1 and
Developmental
Signal for F2**

Increased behavioral/endocrine
reactivity to stress

Maternal
behavior in F1

Neural/behavioral
development of the
next generation

_____

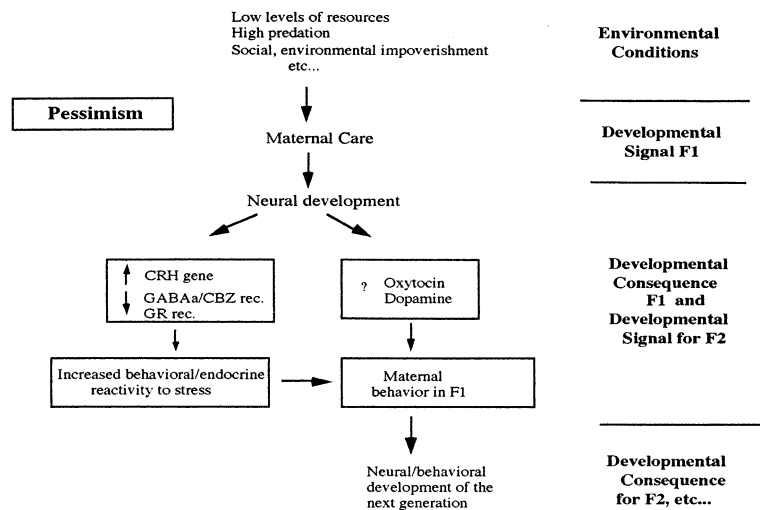**Developmental
Consequence
for F2, etc...**

Figure 2. A schema representing the potential outcomes of the proposed relationship between environmental adversity and infant care. The key feature of this formulation is the hypothesized relationship between fearfulness and maternal behavior (Fleming et al 1988, for a review). Thus, variations in maternal care affect the development of neural systems that mediate stress reactivity, which may then serve to influence maternal behavior. These effects serve to influence the development of the subsequent generation and provide a basis for the transmission of individual differences in stress reactivity from one generation to the next.

behavior clearly depends on decreasing the negative-withdrawal tendency associated with neophobia, and increasing the positive-approach responses. Amygdaloid lesions, which dampen fearful reactions to novelty, also increase maternal responsivity in nulliparous females (Fleming and Anderson 1987). Interestingly, a hormonal regimen that facilitates the expression of maternal behavior in the rat (i.e., exogenous administration of estrogen and progesterone; see Bridges 1994), also reduces the animals fear of novelty (Fleming et al 1989). Such findings may apply to the human condition. Fleming (1988) reported that many factors contribute to the quality of the mother's attitude towards her newborn, but none were correlated more highly than the women's level of anxiety. Mothers who felt depressed and anxious were, not surprisingly, less positive towards their baby (also see Field 1998). Behaviorally, more fearful, anxious mothers, such as the low LG-ABN dams, appear to be less maternally responsive towards their offspring.

Under natural conditions, and the sanctity of the burrow, rat pups have little direct experience with the environment. Instead, conditions such as the scarcity of food, social instability, low dominance status, and so forth, directly affect the status of the mother and thus, maternal care. The effects of these environmental challenges on the development of the pups are then mediated by alterations in maternal care (Figure 2). Variations in maternal care can, thus, serve to transduce an environmental signal to the pups. The environmentally driven alterations in maternal care then influence the development of neural systems that mediate behavioral and HPA responses to stress (Figure 2). These effects can thus serve to increase or decrease stress reactivity in the offspring. We propose that more fearful, anxious animals, such as the low LG-ABN mothers, are therefore more neophobic and lower in maternal

responsivity to pups than are less fearful animals. Hence, these effects serve as the basis for comparable patterns of maternal behavior in the offspring (F1) and for the transmission of these traits to the subsequent generation (F2; see Figure 2).

These individual differences are transmitted to the offspring in terms of effects on the development of neural systems mediating the expression of fearfulness. Perhaps the pivotal finding is that maternal care in infancy regulates the development of central CRF systems, which serve to activate behavioral, endocrine, and autonomic responses to stress. Variations in maternal care in the infant rat also influences the development neural systems, such as glucocorticoid and $GABA_A$ receptor systems, which provide an inhibitory tone over CRF synthesis and release.

In addition, we propose that there are also effects of maternal care on neural systems mediating attraction to pup-related stimuli. We found preliminary evidence for reduced oxytocin receptor levels in the amygdala of the low LG-ABN mothers, as well as alterations in ascending dopamine systems. Both systems have been implicated in the expression of maternal behavior in the rat (see Bridges and Freemark 1995; Pederson 1995; Stern 1997 for reviews). Individual differences in maternal care could, therefore, be derived from early environmental effects on the development of neural systems mediating fearfulness as well as those involved in maternal responsivity. Together, these effects, in turn, provide the basis for stable individual differences in stress reactivity and maternal behavior in the offspring.

Perhaps the most compelling evidence for this process emerges from the studies of Rosenblum and colleagues (see Rosenblum and Andrews 1994 for a review). Bonnet macaque mother–infant dyads were maintained under one of three foraging conditions: low foraging demand (LFD), where food was readily available; high foraging demand

(HFD) where ample food was available, but required long periods of searching; and variable foraging demand (VFD), a mixture of the two conditions on a schedule that did not allow for predictability. At the time that these conditions were imposed, there were no differences in the nature of mother–infant interactions. Following a number of months of these conditions, however, there were highly significant differences in mother–infant interactions. The VFD condition was clearly the most disruptive. Mother–infant conflict increased in the VFD condition. Infants of mothers housed under these conditions were significantly more timid and fearful. These infants showed signs of depression common observed in maternally separated macaque infants, remarkably, even while the infants were in contact with their mothers. As adolescents, the infants reared in the VFD conditions were more fearful and submissive and showed less social play behavior.

More recent studies have demonstrated the effects of these conditions on the development of neurobiologic systems that mediate the organisms behavioral and endocrine/metabolic response to stress. In collaboration with Nemeroff's group (Coplan et al 1996, 1998), Rosenblum showed that, as adults, monkeys reared under VFD conditions showed increased cerebrospiral fluid levels of CRF. Increased central CRF drive would suggest altered noradrenergic and serotonergic responses to stress, and this is exactly what was seen in adolescent VFD-reared animals. It will be interesting to see if these traits are then transmitted to the next generation.

Conclusions

These patterns of transmission likely reflect very adaptive patterns of development. Children inherit not only genes from their parents, but also an environment (West and King 1987). We believe that these are adaptive patterns of development. Under conditions of increased environmental demand, it is commonly in the animal's interest to enhance its behavioral (e.g., vigilance, fearfulness) and endocrine (HPA and metabolic/cardiovascular) responsivity to stress (see "Background and Significance"). These responses promote detection of potential threat, avoidance learning, and metabolic/cardiovascular responses that are essential under the increased demands of the stressor. Since the offspring usually inhabit a niche that is similar to their parents, the transmission of these traits from parent to offspring could serve to be adaptive. It is thus perhaps understandable that parents occupying a highly demanding environment would transmit to their young a high level of stress reactivity. Farrington's (1988) research on young males growing-up in a low socioeconomic status and high crime environment in London, provides an excellent illustration of this point. In this environment, the men that

were most successful in avoiding the pitfalls associated with such a "criminogenic" environment were those that were shy and somewhat timid. Under such conditions, a parental rearing style that favored the development of a greater level of reactivity to threat would be adaptive. The obvious conclusion is that there is no single ideal form of parenting. Different environments demand different traits in the offspring. A final issue concerns the cost of such increased stress reactivity. The shy and timid child in the London slum may be at an advantage with respect to the demands of the immediate environment. The question is whether such traits would later also confer a increased risk for stress-induced illness. We would argue that it does, and that this risk reflects the cost of adaptation to a high level of environmental demand, such as a low socio-economic environment, in early life.

References

Ader R, Grota LJ (1969): Effects of early experience on adrenocortical reactivity. *Physiol Behav* 4:303–305.

Alberts JR (1994): Learning as adaptation of the infant. *Acta Paediatr Suppl* 397:77–85.

Anisman H, Zaharia MD, Meaney MJ, Merali Z (1998): Do early life events permanently alter behavioral and hormonal responses to stressors? *Int J Dev Neurosci* 16:149–164.

Barnett SA (1967): Rats. *Sci Am* 21:79–85.

Baxter JD, Tyrrell JB (1987): The adrenal cortex. In: Felig P, Baxter JD, Broadus AE, Frohman LA, editors, *Endocrinology and Metabolism*. New York: McGraw-Hill, 385–511.

Bell RW, Nitschke W, Gorry TH, Zachman T (1971): Infantile stimulation and ultrasonic signaling: A possible mediator of early handing phenomena. *Dev Psychobiol* 4:181–191.

Berman CM (1990): Intergenerational transmission of maternal rejection rates among free-ranging rhesus monkeys on Cayo Santiago. *Anim Behav* 44:247–258.

Bhatnagar S, Shanks N, Meaney MJ (1995): Hypothalamic–pituitary–adrenal function in handled and nonhandled rats in response to chronic stress. *J Neuroendocrinol* 7:107–119.

Bifulco A, Brown GW, Adler Z (1991): Early sexual abuse and clinical depression in aduult life. *Br J Psychiatry* 159:115–122.

Brady LS, Gold PW, Herkenham M, Lynn AB, Whitfield HW (1992): The antidepressants fluoxetine, idazoxan and phenelzine alter corticotropin-releasing hormone and tyrosine hydroxylase mRNA levels in rat brain: Therapeutic implications. *Brain Res* 572:117–125.

Bridges RS (1994): The role of lactogenic hormones in maternal behavior in female rats. *Acta Paediatr Suppl* 397:33–39.

Bridges RS, Freemark MS (1995): Human placental lactogen infusions into the medial preoptic area stimulate maternal behavior in steroid-primed, nulliparous female rats. *Horm Behav* 29:216–226.

Brindley DN, Rolland Y (1989): Possible connections between stress, diabetes, obesity, hypertension and altered lipoprotein metabolism that may result in atherosclerosis. *Clin Sci* 77: 453–461.

Brown GR, Anderson B (1993): Psychiatric morbidity in adult inpatients with childhood histories of sexual and physical abuse. *Am J Psychiatry* 148:55–61.

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.

Cahill L, McGaugh JL (1998): Mechanisms of emotional arousal and lasting declarative memory. *Trend Neurosci* 21:294–299.

Caldji C, Diorio J, Francis DD, Sharma S, Meaney MJ (in press): Maternal care during infancy regulates the expression of GABAergic receptor subunit expression.

Caldji C, Diorio J, Plotsky PM, Meaney MJ (1999): Multiple central benzodiazepine/GABA$_A$ receptor subunits regulated by maternal behavior in infancy. *Soc Neurosci Abstr* 25:616.

Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ (1998): Maternal care during infancy regulates the development of neural systems mediating the expression of behavioral fearfulness in adulthood in the rat. *Proc Nat Acad Sci U S A* 95:5335–5340.

Calhoun JB (1962): *The Ecology and Sociology of the Norway Rat.* Bethesda, MD: H.E.W. Public Health Service.

Canetti L, Bachar E, Galili-Weisstub E, De-Nour AK, Shalev AY (1997): Parental bonding and mental health in adolescence. *Adolescence* 32:381–394.

Chrousos GP, Gold PW (1992): The concepts of stress and stress system disorders. *JAMA* 267:1244–1252.

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

Coplan JD, Trost RC, Owens MJ, Cooper TB, Gorman JAM, Nemeroff CB, et al (1998): Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch Gen Psychiatry* 55:473–477.

Daly M (1973): Early stimulation of rodents: A critical review of present interpretations. *Br J Psychol* 64:435–460.

DeBellis MD, Chrousos GP, Dorn LD, Burke L, Helmers K, Kling MA, et al (1994): Hypothalamic pituitary adrenal dysregulation in sexually abused girls. *J Clin Endocrinol Metab* 78:249–255.

de Kloet ER (1991): Brain corticosteroid receptor balance and homeostatic control. *Neuroendocrinology* 12:95–164.

Denenberg VH (1963): Early experience and emotional development. *Sci Am* 208:138–146.

Denenberg VH (1964): Critical periods, stimuli input, and emotional reactivity: A theory of infantile stimulation. *Psychol Rev* 71:335–351.

Eisenberg L (1990): The biosocial context of parenting in human families. In: Krasnegor NA, Bridges RS, editors, *Mammalian Parenting.* New York: Oxford University Press, 9–16.

Fairbanks LM (1996): Individual differences in maternal style. *Adv Study Behav* 25:579–611.

Fairbanks LY (1989): Early experience and cross-generational continuity of mother–infant contact in Vervet monkeys. *Dev Psychobiol* 22:669–681.

Farrington DA, Gallagher B, Morley L, St Ledger RJ, West DJ (1988): Are there any successful men from criminogenic backgrounds? *Psychiatry* 51:116–130.

Field T (1998): Maternal depression effects on infants and early interventions. *Prev Med* 27:200–203.

Fleming A, Corter C (1988): Factors influencing maternal responsiveness in humans: Usefulness of an animal model. *Psychoneuroendocrinology* 13:189–212.

Fleming AS (1988): Factors influencing maternal responsiveness in humans: Usefulness of an animal model. *Psychoneuroendocrinology* 13:189–212.

Fleming AS, Anderson V (1987): Affect and nurturance: Mechanisms mediating maternal behavior in two female mammals. *Prog Neuropsychopharmacol Biol Psychiatry* 11:121–127.

Fleming AS, Cheung U, Myhal N, Kessler Z (1989): Effects of maternal hormones on "timidity" and attraction to pup-related odors in female rats. *Physiol Behav* 46:449–453.

Francis D, Liu D, Brake W, Meaney MJ (1999): Variations in maternal care: What exactly does it regulate. *Society for Behavioral Neuroendocrinology Abstract*, #42.

Francis D, Sharma S, Liu D, Pearson D, Plotsky PM, Meaney MJ (1998): Variations in maternal care form the basis for a non-genomic mechanism of inter-generational transmission of individual differences in behavioral and endocrine responses to stress. *Soc Neurosci Abs* 24:452.

Gray JA (1987): *The Psychology of Fear and Stress.* Cambridge, UK: Cambridge University Publications.

Gray TS (1990): The organization and possible function of amygdaloid corticotropin-releasing factor pathways. In: De Souza EB, Nemeroff CB, editors. *Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide.* Boca Raton, FL: CRC Press, 53–68.

Gray TS, Bingaman EW (1996): The amygdala: Corticotropin-releasing factor, steroids, and stress. *Crit Rev Neurobiol* 10:155–168.

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

Hess JL, Denenberg VH, Zarrow MX, Pfeifer WD (1969): Modification of the corticosterone response curve as a function of handling in infancy. *Physiol Behav* 4:109–112.

Higley JD, Haser MF, Suomi SJ, Linnoila M (1991): Nonhuman primate model of alcohol abuse: Effects of early experience, personality and stress on alcohol consumption. *Proc Natl Acad Sci U S A* 88:7261–7265.

Holmes SJ, Robins LN (1987): The influence of childhood disciplinary experience on the development of alcoholism and depression. *J Child Psychol Psychiatry* 28:399–415.

Holmes SJ, Robins LN (1988): The role of parental disciplinary

practices in the development of depression and alcoholism. *Psychiatry* 51:24–36.

Jacobson L, Sapolsky RM (1991): The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenal axis. *Endocrinol Rev* 12:118–134.

Jans J, Woodside BC (1990): Nest temperature: Effects on maternal behavior, pup development, and interactions with handling. *Dev Psychobiol* 23:519–534.

Koegler-Muly SM, Owens MJ, Ervin GN, Kilts CD, Nemeroff CB (1993): Potential corticotropin-releasing factor pathways in the rat brain as determined by bilateral electrolytic lesions of the central amygdaloid nucleus and paraventricular nucleus of the hypothalamus. *J Neuroendocrinol* 5:95–98.

Kraemer GW, Ebert MH, Schmidt DE, McKinney WT (1989): A longitudinal study of the effect of different social rearing conditions on cerebrospinal fluid norepinephrine and biogenic amine metabolites in rhesus monkeys. *Neuropsychopharmacology* 2:175–189.

Ladd CO, Owens MJ, Nemeroff CB (1996): Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology* 137:1212–1218.

Lavicky J, Dunn AJ (1993): Corticotropin-releasing factor stimulates catecholamine release in hypothalamus and prefrontal cortex in freelymoving rats as assessed by microdialysis. *J Neurochem* 60:602–612.

Lee MHS, Williams DI (1974): Changes in licking behaviour of rat mother following handling of young. *Anim Behav* 22:679–681.

Lee MHS, Williams DI (1975): Long term changes in nest condition and pup grouping following handling of rat litters. *Dev Psychobiol* 8:91–95.

Lee Y, Schulkin J, Davis M (1994): Effect of corticosterone on the enhanced acoustic startle reflex by corticotropin-releasing factor. *Brain Res* 666:93–99.

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

Levine S (1962): Plasma-free corticosteroid response to electric shock in rats stimulated in infancy. *Science* 135:795–796.

Levine S (1975): Psychosocial factors in growth and development. In: Levi L, editor. *Society, Stress and Disease*. London: Oxford University Press, 43–50.

Levine S (1994) The ontogeny of the hypothalamic–pituitary–adrenal axis. The influence of maternal factors. *Ann N Y Acad Sci.* 746:275–288.

Levine S, Haltmeyer GC, Karas GG, Denenberg VH (1967): Physiological and behavioral effects of infantile stimulation. *Physiol Behav* 2:55–63.

Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ (in press): The effects of early life events on in vivo release of norepinephrine in the paraventricular nucleus of the hypothalamus and hypothalamic–pituitary–adrenal responses during stress. *J Neuroendocrinol*.

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

Lupien S, Meaney MJ (1998): Stress, glucocorticoids, and hippocampus aging in rat and human. In: Wang E, Snyder S, editors. *Handbook of Human Aging*. New York: Academic Press, 19–50.

Maestripieri D (1998): Parenting styles of abusive mothers in group-living rhesus macaques. *Anim Behav* 55:1–11.

Maestripieri D (1999): Consistency and change in the behavior of rhesus macaque abusive mothers with successive infants. *Dev Psychobiol* 34:29–35.

McCarty R, Gold PE (1996): Catecholamine, stress, and disease: A psychobiological perspective. *Psychosom Med* 58(6):590–597.

McEwen BS (1998): Stress, adaptation, and disease. Allostasis and allostatic load. *Ann N Y Acad Sci* 940:33–44.

McEwen BS, de Leon MJ, Lupien S, Meaney MJ (in press): Corticosteroids, the aging brain and cognition. *Trend Endocrinol Metab.*

McEwen BS, Steller E (1993): Stress and the individual: Mechanisms leading to disease. *Arch Intern Med* 153:2093–2101.

Meaney MJ (in press): Early experience effects on HPA function—a basis for individual differences. In: McEwen BS and Steller E, editors. *Handbook of Physiology: Coping with the Environment*. New York: Oxford University Press.

Meaney MJ, Aitken DH (1985): The effects of early postnatal handling on the development of hippocampal glucocorticoid receptors: Temporal parameters. *Dev Brain Res* 22:301–304.

Meaney MJ, Aitken DH, Sapolsky RM (1991): Environmental regulation of the adrenocortical stress response in female rats and its implications for individual differences in aging. *Neurobiol Aging* 12:31–38.

Meaney MJ, Aitken DH, Sharma S, Viau V, Sarrieau A (1989): Postnatal handling increases hippocampal type II, glucocorticoid receptors and enhances adrenocortical negative-feedback efficacy in the rat. *Neuroendocrinology* 51:597–604.

Meaney MJ, Diorio J, Widdowson J, LaPlante P, Caldji C, Seckl JR, et al (1996): Early environmental regulation of forebrain glucocorticoid receptor gene expression: Implications for adrenocortical responses to stress. *Dev Neurosci* 18:49–72.

Miller L, Kramer R, Warner V, Wickramaratne P, Weissman M (1997): Intergenerational transmission of parental bonding among women. *J Am Acad Child Adolesc Psychiatry* 36:1134–1139.

Moga MM, Gray TS (1989): Evidence for corticotropin-releasing factor, neurotensin, and somatostatin in the neural pathway from the central nucleus of the amygdala to the parabranchial nucleus. *J Comp Neurol* 241:275–284.

Moore CL, Lux BA (1998): Effects of lactation on sodium intake in fischer-344 and Long-Evans rats. *Dev Psychobiol* 32:51–56.

Moore CL, Wong L, Daum MC, Leclair OU (1997): Mother–infant interactions in two strains of rats: Implications for dissociating mechanism and function of a maternal pattern. *Dev Psychobiol* 30:301–312.

Munck A, Guyre PM, Holbrook NJ (1984): Physiological functions of glucocorticoids in stress and their relations to pharmacological actions. *Endocr Rev* 5:25–44.

Nemeroff CB (1996): The corticotropin-releasing factor (CRF) hypothesis of depression: New findings and new directions. *Mol Psychiatry* 1:336–342.

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

O'Donnell D, Larocque S, Seckl JR, Meaney MJ (1994): Postnatal handling alters glucocorticoid, but not mineralocorticoid mRNA expression in adult rats. *Mol Brain Res* 26:242–248.

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

Pacak K, Palkovits M, Kopin I, Goldstein DS (1995): Stress-induced norepinephrine release in hypothalamic paraventricular nucleus and pituitary-adrenrocortical and sympatho-adrenal activity: In vivo microdialysis studies. *Front Neuroendocrinol* 16:89–150.

Parker G (1981): Parental representations of patients with anxiety neurosis. *Acta Psychiatr Scand* 63:33–36.

Patchev VK, Montkowski A, Rouskova D, Koranyi L, Holsboer F, Almeida OF (1997): Neonatal treatment of rats with the neuroactive steroid tetrahydordeoxycorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. *J Clin Invest* 99:962–966.

Pearson D, Sharma S, Plotsky PM, Pfaus JG, Meaney MJ (1997): The effect of postnatal environment on stress-induced changes in hippocampal FOS-like immunoreactivity in adult rats. *Soc Neurosci Abst* 23:1849.

Pedersen CA (1995): Oxytocin control of maternal behavior. Regulation by sex steroids and offspring stimuli. *Ann N Y Acad Sci* 807:126–145.

Plotsky PM (1991): Pathways to the secretion of adrenocorticotropin: A view from the portal. *J Neuroendocrinol* 3:1–9.

Plotsky PM, Cunningham ET, Widmaier EP (1989): Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. *Endocr Rev* 10:437–458.

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. *Mol Brain Res* 18:195–200.

Plotsky PM, Meaney MJ (in press): Early environment alters hypothalamic–pituitary–adrenal responses to a wide range of stressful stimuli. *Proc Natl Acad Sci USA*.

Rivier C, Plotsky PM (1986): Mediation by corticotropin-releasing factor of adenohypophysial hormone secretion. *Ann Rev Physiol* 48:475–489.

Rosenblatt JS (1994): Psychobiology of maternal behavior: Contribution to the clinical understanding of maternal behavior among humans. *Acta Paediatr Suppl* 397:3–8.

Rosenblum LA, Andrews MW (1994): Influences of environmental demand on maternal behavior and infant development. *Acta Paediatr Suppl* 397:57–63.

Rosenblum LA, Coplan JD, Friedman S, Bassoff T, Gorman JM, Andrews MW (in press): Adverse early experiences affect noradrenergic and serotonergic functioning in adult primates. *Biol Psychiatry*.

Rowe WB, Nicot A, Sharma S, Gully D, Walker CD, Rostene WB, et al (1997): Central administration of the neurotensin receptor antagonist, SR48692, modulates diurnal and stress-related hypothalamic-pituitary-adrenal activity. *Neuroendocrinol* 66:75–85.

Russak LG, Schwartz GE (1997): Feelings of parental care predict health status in midlife: A 35-year follow-up of the Harvard Mastery of Stress Study. *J Behav Med* 20:1–11.

Sapolsky RM (1994): The physiological relevance of glucocorticoid endangerment of the hippocampus. *Ann N Y Acad Sci* 746:294–304.

Sarrieau A, Sharma S, Meaney MJ (1988): Postnatal development and environmental regulation of hippocampal glucocorticoid and mineralocorticoid receptors in the rat. *Dev Brain Res* 43:158–162.

Scarr S, McCarthy K (1983): How people make their own environments: A theory of genotype–environment effects. *Child Dev* 54:424–435.

Schanberg SM, Evoniuk G, Kuhn CM (1984): Tactile and nutritional aspects of maternal care: Specific regulators of neuroendocrine function and cellular development. *Proc Soc Exp Biol Med* 175:135–146.

Schulkin J (1999): Corticotropin-releasing hormone signals adversity in both the placenta and the brain: Regulation by glucocorticoids and allostatic overload. *J Endocrinol* 161:349–356.

Schulkin J, Gold PW, McEwen BS (1998): Induction of corticotropin-releasing hormone gene expression by glucocorticoids: Implication for understanding the states of fear and anxiety and allostatic load. *Psychoneuroendocrinology* 23:219–243.

Seckl JR, Meaney MJ (1994): Early life events and later development of ischaemic heart disease. *Lancet* 342:1236.

Stern JM (1997): Offspring-induced nurturance: Animal–human parallels. *Dev Psychobiol* 31:19–37.

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

Valentino RJ, Curtis AL, Page ME, Pavcovich LA, Florin-Lechner SM (1988): Activation of the locus cereulus brain noradrenergic system during stress: Circuitry, consequences, and regultion. *Adv Pharmacol* 42:781–784.

Valentino RJ, Foote SL (1988): Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. *J Neurosci* 8:1016–1025.

van Bockstaele EJ, Colago EEO, Valentino RJ (1996): Corticotropin-releasing factor-containing axon terminals synapse onto catecholamine dendrites and may presynaptically modulate other afferents in the rostral pole of the nucleus locus coeruleus in the rat brain. *J Comp Neurol* 364:523–534.

Van der Kooy D, Koda LY, McGinty JF, Gerfen CR, Bloom FE (1984): The organization of projections from the cortex, amygdala and hypothalamus to the nucleus of the solitary tract in rat. *J Comp Neurol* 224:1–24.

Viau V, Sharma S, Plotsky PM, Meaney MJ (1993): Increased plasma ACTH responses to stress in nonhandled compared with handled rats require basal levels of corticosterone and are associated with increased levels of ACTH secretagogues in the median eminence. *J Neurosci* 13:1097–1105.

West MJ, King AP (1987): Settling nature and nurture into an ontogenetic niche. *Dev Psychobiol* 20:549–562.

Wilson MA (1996): GABA physiology: Modulation by benzodiazepines and hormones. *Crit Rev Neurobiol* 10:1–37.

Zaharia MD, Shanks N, Meaney MJ, Anisman H (1996): The effects of postnatal handling on Morris water maze acquisition in different strains of mice. *Psychopharmacol* 128:227–239.

Zarrow MX, Campbell PS, Denenberg VH (1972): Handling in infancy: Increased levels of the hypothalamic corticotropin releasing factor (CRF) following exposure to a novel situation. *Proc Soc Exp Biol Med* 356:141–143.