

MATERNAL CARE, GENE EXPRESSION, AND THE TRANSMISSION OF INDIVIDUAL DIFFERENCES IN STRESS REACTIVITY ACROSS GENERATIONS

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■ **Abstract** Naturally occurring variations in maternal care alter the expression of genes that regulate behavioral and endocrine responses to stress, as well as hippocampal synaptic development. These effects form the basis for the development of stable, individual differences in stress reactivity and certain forms of cognition. Maternal care also influences the maternal behavior of female offspring, an effect that appears to be related to oxytocin receptor gene expression, and which forms the basis for the intergenerational transmission of individual differences in stress reactivity. Patterns of maternal care that increase stress reactivity in offspring are enhanced by stressors imposed on the mother. These findings provide evidence for the importance of parental care as a mediator of the effects of environmental adversity on neural development.

PARENTAL CARE AND THE HEALTH OF OFFSPRING

The quality of family life influences the development of individual differences in vulnerability throughout life to illness. As adults, victims of childhood physical or sexual abuse are at considerably greater risk for mental illness, as well as for obesity, diabetes, and heart disease (e.g. Bifulco et al 1991, Brown & Anderson 1993, McCauley et al 1997, Felitti et al 1998). Children need not be beaten to be compromised. Persistent emotional neglect, family conflict, and conditions of harsh, inconsistent discipline all serve to compromise growth (e.g. Montgomery et al 1997) and intellectual development (Ammerman et al 1986, Trickett & McBride-Chang 1995) and to increase the risk for adult obesity (Lissau & Sorensen 1994), depression, and anxiety disorders (Holmes & Robbins 1987, 1988; Gottman 1998) to a level comparable to that for abuse.

More subtle relationships also exist. Low scores on measures of parental bonding, reflecting cold, distant parent-child relationships are associated with a significantly increased risk of depression and anxiety in later life (e.g. Canetti et al 1997, Parker 1981). And again, the risk is not unique to mental health. Russak & Schwartz (1997) found that by midlife, those individuals who, as undergraduate students, rated their relationships with parents as cold and detached had a fourfold greater risk of chronic illness, including depression and alcoholism as well as heart disease and diabetes. The sword cuts both ways. Family life can also serve as a source of resilience in the face of chronic stress (Rutter 1979). Thus, warm, nurturing families tend to promote resistance to stress and to diminish vulnerability to stress-induced illness (Smith & Prior 1995).

Parental factors also serve to mediate the effects of environmental adversity on development. For example, the effects of poverty on emotional and cognitive development are mediated by parental factors to the extent that if such factors are controlled, there is no discernible effect of poverty on child development (Eisenberg & Earls 1975, Conger et al 1994, McLloyd 1998). Moreover, treatment outcomes associated with early intervention programs are routinely correlated with changes in parental behavior: In cases where parental behavior proves resistant to change, treatment outcomes for the children are seriously limited.

A critical question concerns the mechanisms that mediate these enduring parental influences on the health of offspring. The relationship between early life events and health in adulthood appears to be, in part, mediated by parental influences on the development of neural systems that underlie the expression of behavioral and endocrine responses to stress (Seckl & Meaney 1994, Nemeroff 1996, Sroufe 1997, Francis & Meaney 1999, Francis et al 1999a, Heim et al 2001). Physical and sexual abuse in early life, for example, increases endocrine and autonomic responses to stress in adulthood (DeBellis et al 1994, Heim et al 2001). There are two critical assumptions here: First, that prolonged activation of neural and hormonal responses to stress can promote illness; and second, that early environmental events influence the development of these responses. There is strong evidence in favor of both ideas.

RESPONSES TO STRESS

Stress is a risk factor for a variety of diseases, ranging from autoimmune disorders to mental illness. It is ironic that the pathways by which stressful events promote the development of such divergent forms of illness involve the same hormones that ensure survival during a period of stress (Chrousos & Gold 1992, McEwen & Stellar 1993, McEwen 1998). These effects can, to some extent, be understood in terms of the normal set of adaptive responses elicited by stressors (Dallman et al 1987, 1995; Chrousos & Gold 1992; McEwen & Stellar 1993; De Kloet et al 1998). The increased sympathoadrenal release of catecholamines, primarily adrenaline and noradrenaline, as well as the adrenal glucocorticoids, orchestrate a move to catabolism, mobilizing lipid and glucose reserves, and to insulin antagonism

(Munck et al 1984, Baxter & Tyrrell 1987, Brindley & Rolland 1989, Dallman et al 1995). The increase in circulating levels of catecholamines and glucocorticoids also promotes increased cardiovascular tone. These actions serve to increase the availability and distribution of energy substrates. Although these responses serve to meet the metabolic demands posed by the stressor, prolonged exposure to elevated levels of these "stress hormones" can promote insulin resistance, hypertension, hyperlipidemia, hypercholesterolemia, abdominal fat deposition, and an increased risk of arterial damage, all of which are associated with an increased risk for heart disease (Brindley & Rolland 1989, Rosmond et al 1995).

There are also cognitive responses to stressors that include systems that mediate attentional processes, as well as learning and memory (Arnsten 1998). During stress, individuals become hypervigilant; the level of attention directed to the surrounding environment is increased at the expense of effortful concentration on tasks that are not essential for survival. As a result of these changes in attentional processes, as well as of the effects of glucocorticoids on such relevant brain structures as the hippocampus, episodic memory capacity is diminished during periods of stress (Landfield & Pitler 1984, Joels & De Kloet 1989, Diamond et al 1992, Starkman et al 1992, Sapolsky 1992, Lupien et al 1998, Lupien & Meaney 1998, De Kloet et al 1998, McEwen et al 1999). At the same time, glucocorticoids act on such areas of the brain as the amygdala to enhance learning and memory for emotionally salient events (e.g. Davis et al 1997, Quirarte et al 1997, Pitkanen et al 1998, Cahill & McGaugh 1998). Stress also provokes altered emotional states: Feelings of apprehension and fear predominate during a stressful experience. Although these responses are highly adaptive, chronic activation of these systems can promote the emergence of specific forms of cognitive impairments, states of anxiety and dysphoria, sleep disorders, etc (Koob et al 1994, Nemeroff 1996, Rosen & Schulkin 1998).

Herein lies the dilemma: The same responses that permit survival during stress can ultimately promote disease. Consequently, individual differences in endocrine and sympathetic responses to stress can serve as a source of vulnerability (or resistance) to pathology over a life span (McEwen & Stellar 1993, Chrousos & Gold 1992). In human and nonhuman populations, individuals who show exaggerated hypothalamic-pituitary-adrenal (HPA) and sympathetic responses to stress are at increased risk for a variety of disorders, including heart disease, diabetes, anxiety, depression, and drug addiction.

Corticotropin-Releasing Factor Systems

Central corticotropin-releasing factor (CRF) systems furnish the critical signal for the activation of behavioral, emotional, autonomic, and endocrine responses to stressors. There are two major CRF pathways regulating the expression of these stress responses. First, a CRF pathway from the parvocellular regions of the paraventricular nucleus of the hypothalamus (PVN_h) to the hypophysial-portal system of the anterior pituitary serves as the principal mechanism for the transduction of

a neural signal into a pituitary-adrenal response (Rivier & Plotsky 1986, Plotsky 1991, Antoni 1993, Whitnall 1993, Pacak et al 1995). In responses to stressors, CRF, as well as such cosecretagogues as arginine vasopressin, are released from PVN_h neurons into the portal blood supply of the anterior pituitary, where it stimulates the synthesis and release of adrenocorticotropin hormone (ACTH). Pituitary ACTH, in turn, causes the release of glucocorticoids from the adrenal gland.

CRF neurons in the central nucleus of the amygdala project directly 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. Thus, intracerebroventricular infusion of CRF increases extracellular noradrenaline levels (Lavicky & Dunn 1993, Emoto et al 1993, Page & Valentino 1994, Valentino et al 1998). The amygdaloid CRF projection to the locus coeruleus (Moga & Gray 1989, Koegler-Muly et al 1993, van Bockstaele et al 1996, Gray & Bingaman 1996, Valentino et al 1998) is also critical for the expression of behavioral responses to stress: Microinjections of CRF receptor antagonists into the locus coeruleus attenuate fear-related behaviors (Butler et al 1990, Liang et al 1992, Sweirgel et al 1993, Koob et al 1994, Schulkin et al 1994, Bakshi et al 2000) and are emerging as a major target for drug development in the treatment of affective disorders. In contrast, CRF overproduction is associated with increased fearfulness (e.g. Stenzel-Poore et al 1994). Hence, the CRF neurons in the PVN_h and the central n. of the amygdala serve as important mediators of both behavioral and endocrine responses to stress. It is not surprising that chronically increased CRF levels have been associated with serious mood disorders (Chrousos & Gold 1992, Nemeroff 1996).

These findings provide an understanding of how stress can influence health. Yet the influence of stress can only be fully appreciated when we factor into the equation some appreciation of the individual's response to stress. After all, 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 research on the development of psychopathology focuses on the role of early life events in determining individual differences in vulnerability to stress. This hypothesis rests on the assumption that chronic activation of central and endocrine stress responses can promote illness (see references cited above). Thus, early life events that increase stress reactivity result in a greater vulnerability to stress-induced illness over a life span.

ENVIRONMENTAL REGULATION OF HPA AND BEHAVIORAL RESPONSES TO STRESS

Postnatal Handling Studies

Perhaps the strongest evidence for the environmental regulation of the development of responses to stress is from postnatal handling research with rodents. Handling involves a brief (i.e. 3–15 min), daily period of separation of the pup from the

mother for the first few weeks of life and results in decreased stress reactivity in adulthood (Levine 1957, 1962; Levine et al 1967; Ader & Grotta 1969; Hess et al 1969; Zarrow et al 1972; Meaney et al 1989; Viau et al 1993; Bhatnagar et al 1995). As adults, rats handled neonatally show decreased fearfulness and more modest HPA responses to stress; such effects are apparent in animals tested as late as 26 months of age (Meaney et al 1988, 1992).

The handling effects on the development of HPA responses to stress have important consequences for health. Glucocorticoid levels often rise with age in rats and are associated with hippocampal degeneration and the emergence of learning and memory deficits (Landfield et al 1981, Landfield & Pitler 1984, Sapolsky et al 1984, Issa et al 1990). Such age-related increases in basal and stress-induced pituitary-adrenal activity is significantly less apparent in the handled animals, and thus these animals show little evidence of hippocampal aging (Meaney et al 1988, 1991). Likewise, handled animals also show more modest stress-induced suppression of immune function compared with nonhandled rats (Bhatnagar et al 1996).

Such findings have tempted researchers to believe that the handled animals are in some ways hardier than nonhandled animals. However, this misses the point. Handled animals are not better adapted than nonhandled animals, they are simply different. The environmental context then serves to determine the adaptive value of increased or decreased stress reactivity. In the examples cited above, it would appear that the handled animals were at some advantage. But this is not universal. Laban et al (1995) found that nonhandled animals were more resistant to the induction of experimental allergic encephalomyelitis (EAE) than were handled animals. Normally, glucocorticoids are protective against the development of EAE, which can be fatal (Mason 1991). Adrenalectomized animals, for example, rarely survive EAE. Hence, the increased HPA responsiveness of nonhandled animals appears to have rendered these animals some advantage. The cost of such resistance is increased vulnerability to glucocorticoid-induced illness, but it is not difficult to imagine a scenario whereby such a cost is an acceptable trade-off. The point here is that these animals differ in stress reactivity and such differences are derived from early life experience. The critical question concerns the mechanisms that mediated these differences in stress reactivity.

Considering the importance of the CRF systems to both behavioral and HPA responses to stress, it is probably not surprising that these systems are critical targets for the handling effect on stress reactivity. Compared with nonhandled rats, adult animals exposed to postnatal handling show decreased CRF mRNA expression in the PVN_h and the central n. of the amygdala (Plotsky & Meaney 1993; Viau et al 1993; PM Plotsky, C Caldji, S Sharma S, MJ Meaney, submitted for publication), decreased CRF content in the locus coeruleus, and decreased CRF receptor levels in the locus coeruleus. Together, these findings suggest that in the handled animals, there would be decreased CRF-induced activation of the locus coeruleus during stress. At least two recent findings are consistent with this idea. By comparison to nonhandled rats, acute stress in handled animals produces (a) a smaller stress-induced increase in cFOS immunoreactivity (ir) neurons in the

locus coeruleus (Pearson et al 1997) and (b) more modest increases in extracellular noradrenaline levels in the PVN_h (Liu et al 2000). We propose that postnatal handling can decrease the expression of behavioral responses to stress by altering the development of the central n. of the amygdala–locus coeruleus CRF system.

Postnatal handling also affects the development of neural systems that regulate CRF gene expression. Levels of CRF mRNA and protein in PVN_h neurons are subject to inhibitory regulation via glucocorticoid negative feedback (Dallman et al 1987, 1993; De Kloet 1991). Handled rats show increased negative feedback sensitivity to glucocorticoids (Meaney et al 1989, Viau et al 1993). This effect, in turn, is related to the increased glucocorticoid receptor expression in the hippocampus and frontal cortex (Meaney et al 1985, 1989; Sarrieau et al 1988; Viau et al 1993; O'Donnell et al 1994), regions known to mediate the inhibitory effects of glucocorticoids over CRF synthesis in PVN_h neurons (see de Kloet 1991, Jacobson & Sapolsky 1991, Diorio et al 1993, De Kloet et al 1998). The alterations in glucocorticoid receptor expression are a critical feature for the effect of the early environment on negative feedback sensitivity and HPA responses to stress; reversing the differences in hippocampal glucocorticoid receptor levels eliminates the differences in HPA responses to stress between handled and nonhandled animals (Meaney et al 1989).

CRF activity within the amygdaloid–locus coeruleus pathway is subject to GABAergic inhibition (Owens et al 1991, deBoer et al 1992). It is interesting that handled rats also show increased GABAA and central benzodiazepine (CBZ) receptor levels in the noradrenergic cell body regions of the locus coeruleus and the n. tractus solitarius, as well as in the basolateral and central n. of the amygdala. These effects were associated with increased expression of the mRNA for the $\gamma 2$ subunit of the GABAA receptor, which encodes for the CBZ site. Handled animals also showed increased levels of mRNA encoding for the $\alpha 1$ subunit of the GABAA receptor. These findings suggest that the composition of the GABAA receptor complex in brain regions that regulate stress reactivity is influenced by early life events. Handling increases $\alpha 1$ and $\gamma 2$ subunit expression (Caldji et al 1999, 2000a,b), and more importantly, this profile is associated with increased GABA binding (see Wilson 1996, Mehta & Ticku 1999). It is interesting that in humans, individual differences in CBZ receptor sensitivity are associated with vulnerability for anxiety disorders (e.g. Glue et al 1995).

Together, the effects of handling on glucocorticoid and GABAA/CBZ receptor gene expression could serve to dampen CRF synthesis and release, and to decrease the effect of CRF at critical target sites, such as the locus coeruleus. This model provides a reasonable working hypothesis for the mechanisms underlying the handling effect on endocrine and behavioral responses to stress.

Maternal Separation and Responses to Stress

The handling procedure involves briefly separating the pup from the mother for a period of ~15 min. In the course of normal mother-pup interactions, the dam is regularly away from the nest, and the pups, for periods of 20–30 min (Jans &

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 privation of maternal care? Plotsky & Meaney (1993) studied adult animals that were separated from their mothers once per day for 180 min from days 2–14 of life. This manipulation was based on the observation of Calhoun (1962) that in seminaturalistic conditions, subordinate females were often obligated to locate nests at some distance from food and water sources, resulting in periods of separation from their pups that extended for as long as 2–3 h.

The effects of maternal separation on stress reactivity were precisely the opposite of those associated with postnatal handling. As adults, animals exposed to repeated periods of maternal separation showed significantly increased pituitary-adrenal responses to acute stress (Plotsky & Meaney 1993, Liu et al 2000). Maternal separation also resulted in decreased glucocorticoid receptor binding in the hippocampus, hypothalamus, and frontal cortex and resulted in blunted negative feedback sensitivity. As expected, adult animals exposed to maternal separation as neonates also exhibited a marked increase in hypothalamic CRF mRNA and CRF peptide content.

Maternal separation was also associated with (a) a twofold increase in CRF mRNA levels in the central n. of the amygdala, (b) increased CRF-like immunoreactivity at the level of the amygdala, the locus coeruleus, and the neighboring parabrachial nucleus, and (c) increased CRF receptor levels in the locus coeruleus and the raphé nucleus (Ladd et al 1996; PM Plotsky, C Caldji, S Sharma, MJ Meaney, submitted for publication). These findings suggest that maternal separation-induced changes in CRF systems might regulate both noradrenergic and serotonergic responses to stress [for comparable findings in nonhuman primates, see Kraemer et al (1989), Higley et al (1991), Suomi (1997)]. Indeed, we found that PVN_h levels of noradrenaline during stress were elevated in maternal separation animals (Liu et al 2000). Predictably, the maternal separation animals were highly fearful in behavioral tests of novelty (see Caldji et al 2000b). These effects involved reduced exploration or feeding in a novel environment, and an increased acoustic startle responsivity, all of which are mediated, in part at least, by CRF effects on noradrenergic release (Kalin 1985, Dunn & Berridge 1990, Koob et al 1994, Nemeroff 1996, Valentino et al 1998).

WHAT ARE THE CRITICAL FEATURES OF THESE ENVIRONMENTAL MANIPULATIONS?

The decreased mother-pup contact resulting from extended periods of maternal separation is likely to be critical for the effects of this procedure on 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 indeed critical under normal conditions, then what are the relevant features of mother-pup interactions, and how do they influence neural development?

We examined this question by attempting to define naturally occurring variations in maternal behavior over the first 8 days after birth through the simple albeit time-consuming observation of mother-pup interactions in normally reared animals. There was considerable variation in two forms of maternal behavior—licking/grooming of pups and arched-back nursing (Stern 1997). Licking/grooming included both body as well as anogenital licking. 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. Although common, it is not the only posture from which dams nurse. A blanket posture, where the mother is almost lying on the suckling pups, represents a more relaxed version of the arched-back position. As you can imagine, it provides substantially less opportunity for such movements 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. It is interesting that the frequency of licking/grooming and arched-back nursing was highly correlated ($r = +0.91$) across animals, and thus we were able to define mothers according to both behaviors: high or low licking/grooming (LG)–arched-back nursing (ABN) mothers. For the sake of most of the studies described here, high and low LG-ABN mothers were identified as females whose scores on both measures were ± 1 SD above (high) or below (low) the mean for their cohort. It is important that high and low LG-ABN mothers do not differ in the amount of contact time with pups; differences in the frequency of LG or ABN do not occur simply as a function of time in contact with pups. The results of three independent studies have failed to reveal any relationship between the frequency of LG or ABN and either litter size or gender composition (for all, $r < 0.10$). The latter is an important consideration because it has been reported (e.g. Moore 1995) that male pups are licked more frequently than females. However, this refers only to anogenital licking. Our studies measure both anogenital and body licking and focus on the first week of life. The gender differences in anogenital LG reported by Moore & Power (1986) appear only later in development, toward the second week of life. It is important that both groups raise a comparable number of pups to weaning, and there are no differences in the weaning weights of the pups, which suggests an adequate level of maternal care across the groups. These findings also suggest that we are examining the consequences of variations in maternal care that occur within a normal range.

The differences in maternal behavior in the high and low LG-ABN mothers were not unique to the first litter (Francis, Champagne & Meaney, unpublished). Across dams there was a correlation of $+0.84$ between the licking/grooming of the first and second litters and a correlation of $+0.72$ between the licking/grooming scores for the first and third litters. Thus, the individual differences in maternal behavior are rather stable. These findings are comparable to those of primate studies in which individual differences in maternal behavior remained consistent across infants (e.g. Fairbanks 1996).

The critical question, of course, concerns the potential consequences of these differences in maternal behavior for the development of behavioral and neuroendocrine responses to stress. As adults, the offspring of high LG-ABN mothers showed reduced plasma ACTH and corticosterone responses to acute stress in comparison to adult offspring of low LG-ABN mothers. The high LG-ABN offspring also showed significantly increased hippocampal glucocorticoid receptor mRNA expression, enhanced glucocorticoid negative feedback sensitivity, and decreased hypothalamic CRH mRNA levels. Moreover, the magnitude of the corticosterone response to acute stress was significantly correlated with the frequency of both maternal LG ($r = -0.61$) and ABN ($r = -0.64$) during the first week of life, as was the level of hippocampal glucocorticoid receptor mRNA and hypothalamic CRH mRNA expression (for all, $r > 0.70$) (Liu et al 1997).

The offspring of the high and low LG-ABN mothers also differed in behavioral responses to novelty (Caldji et al 1998). As adults, offspring of high LG-ABN mothers showed decreased startle responses, increased open-field exploration, and shorter latencies to eat food provided in a novel environment. The offspring of high LG-ABN mothers also showed decreased CRF receptor levels in the locus coeruleus, increased GABAA and CBZ receptor levels in the basolateral and central n. of the amygdala, as well as in the locus coeruleus (Caldji et al 1998), and decreased CRF mRNA expression in the central nucleus of the amygdala (DD Francis, D Diorio & MJ Meaney, unpublished data). Predictably, stress-induced increases in PVN_H levels of noradrenaline were significantly higher in the offspring of low LG-ABN mothers (Caldji et al 1998).

The adult offspring of high LG-ABN mothers also showed significantly higher levels of GABAA and CBZ receptor binding in the basolateral and central n. of the amygdala, as well as in the locus coeruleus. These findings provide a mechanism for increased GABAergic inhibition of amygdala-locus coeruleus activity. A series of *in situ* hybridization studies have illustrated the molecular mechanism for these differences in receptor binding and suggest that variations in maternal care might actually permanently alter the subunit composition of the GABAA receptor complex in the offspring. The offspring of high LG-ABN mothers show increased levels of the mRNAs for the $\gamma 1$ and $\gamma 2$ subunits, which contribute to the formation of a functional CBZ binding site. Such differences are not unique to the γ subunits. Levels of mRNA for the $\alpha 1$ subunit of the GABAA/CBZ receptor complex are significantly higher in the amygdala and locus coeruleus of high compared with low LG-ABN offspring. The $\alpha 1$ subunit appears to confer higher affinity for GABA, providing the most efficient form of the GABAA receptor complex, through increased receptor affinity for GABA. The adult offspring of low LG-ABN mothers actually show increased expression of the mRNAs for the $\alpha 3$ and $\alpha 4$ subunits in the amygdala and the locus coeruleus. It is interesting that GABAA/CBZ receptors composed of the $\alpha 3$ and $\alpha 4$ subunits show a reduced affinity for GABA compared with the $\alpha 1$ subunit. Moreover, the $\alpha 4$ subunit does not contribute to the formation of a CBZ receptor site.

These differences in GABAA receptor subunit expression are also reflected in the CBZ receptor binding. Although the $\alpha 3$ subunit contributes to the formation of

a BZ receptor binding site, those sites are of the type II rather than type I variety (Hadingham et al 1993). [³H]zolpidem can be used to distinguish type I and type II BZ receptor sites because this radioligand has little affinity for the type II receptor (e.g. Arbilla et al 1986). The previously reported differences in BZ receptor binding capacity between the adult offspring of high compared with low LG-ABN mothers lie in the density of type I sites: Differences in [³H]zolpidem binding map onto, and in fact exceed, those observed using [³H]flunitrazepam, which labels both type I and type II receptor sites. It appears as though the $\alpha 1$ subunit alone produces a type I BZ receptor site. GABAA receptor complexes containing $\alpha 3$ subunit display type II BZ receptor pharmacology. The $\alpha 4$ subunit, as mentioned above, does not produce a BZ receptor type of either variety (e.g. Khan et al 1996).

Thus, variations in the subunit profiles in both groups actively contribute to the differences in type I CBZ and GABAA receptor binding observed in the offspring of high and low LG-ABN mothers. These differences in subunit expression are tissue specific; no such differences are apparent in the hippocampus, hypothalamus, or cortex. Thus, differences in GABAA/CBZ receptor binding are due not simply to a deficit in subunit expression in the offspring of low LG-ABN mothers, but also to an apparently active attempt to maintain a specific GABAA/CBZ receptor profile in selected brain regions. Maternal care during the first week of life permanently alters subunit expression and, thus, GABAA/CBZ receptor composition in adulthood in brain regions that regulate stress reactivity.

Summary

It is interesting that postnatal handling increases maternal LG and ABN, whereas maternal separation has precisely the opposite effect (Liu et al 1997; DD Francis & MJ Meaney, unpublished data). These findings support the long-held belief (Levine 1975, Smotherman & Bell 1980) that the effects of such early environmental manipulations are in fact mediated by alterations in maternal behavior. Together, the results of these studies suggest that the behavior of a mother toward her offspring can “program” behavioral and neuroendocrine responses to stress in adulthood. These effects are associated with sustained changes in the expression of genes in brain regions that mediate responses to stress and form the basis for stable differences between individuals in stress reactivity. These findings provide a potential mechanism for the influence of parental care on vulnerability/resistance to stress-induced illness over a life span.

THE INTERGENERATIONAL TRANSMISSION OF INDIVIDUAL DIFFERENCES IN MATERNAL CARE TO THE OFFSPRING

Individual differences in behavioral and neuroendocrine responses to stress in rats are, in part, derived from naturally occurring variations in maternal care. Such effects might serve as a possible mechanism by which selected traits are

transmitted from one generation to another. Indeed, low LG-ABN mothers are more fearful and show increased HPA responses to stress compared with high LG-ABN dams (Francis et al 2000). Individual differences in stress reactivity are apparently transmitted across generations: Fearful mothers beget more stress-reactive offspring. Likewise, as adults, the female offspring of high LG-ABN mothers show significantly more LG and ABN nursing than did female offspring of low LG-ABN mothers (Francis et al 1999b). Hence, the differences in maternal behavior and stress reactivity are transmitted from one generation to the next.

The obvious question is whether the transmission of these traits occurs only as a function of genomic-based inheritance. If this is the case, then the differences in maternal behavior may be simply an epiphenomena and not causally related to the development of individual differences in behavioral and neuroendocrine responses to stress or to maternal behavior. The issue here is not one of inheritance, that much seems clear. The question concerns the mode of inheritance.

The results of recent studies provide evidence for a nongenomic transmission of individual differences in stress reactivity and maternal behavior (Francis et al 1999b). One study involved a reciprocal cross-fostering of the offspring of low and high LG-ABN mothers. The primary concern here was that the wholesale fostering of litters between mothers is known to affect maternal behavior (Maccari et al 1995). In order to avert this problem and maintain the original character of the host litter, no more than 2 of 12 pups were fostered into or from any one litter (McCarty & Lee 1996). The critical groups of interest are the biological offspring of low LG-ABN mothers fostered onto high LG-ABN dams, and vice versa. The limited cross-fostering design did not result in any effect on group differences in maternal behavior. Hence, the frequency of pup LG and ABN across all groups of high LG-ABN mothers was significantly higher than that for any of the low LG-ABN dams, regardless of litter composition.

The results of the behavioral studies are consistent with the idea that variations in maternal care are causally related to individual differences in the behavior of the offspring. The biological offspring of low LG-ABN dams reared by high LG-ABN mothers were significantly less fearful under conditions of novelty than were the offspring reared by low LG-ABN mothers, including the biological offspring of high LG-ABN mothers. A separate group of female offspring were then mated, allowed to give birth, and observed for differences in maternal behavior. The effect on maternal behavior followed the same pattern as that for differences in fearfulness. As adults, the female offspring of low LG-ABN dams reared by high LG-ABN mothers did not differ from normal, high LG/ABN offspring in the frequency of pup LG or ABN. The frequency of LG and ABN in animals reared by high LG-ABN mothers was significantly higher than in any of the low LG-ABN groups, and again this included female pups originally born to high LG-ABN mothers but reared by low LG-ABN dams. Individual differences in fearfulness or maternal behavior mapped onto those of the rearing mother rather than the biological mother.

Francis et al (1999b) also addressed this question using a variation of the study described above in which the female offspring of high and low LG-ABN mothers

were mated. The pups of female offspring were then either handled or nonhandled during the first 2 weeks of life. Again, the offspring of high LG-ABN mothers showed significantly more LG and ABN of pups than did the offspring of low LG-ABN mothers. Handling the pups of these mothers, as expected (see above), increased maternal LG and ABN. It is interesting that handling affected the maternal behavior only of the female offspring of low LG-ABN mothers. Low LG-ABN mothers with handled pups showed significantly more LG and ABN of pups than did the low LG/ABN-derived mothers of nonhandled pups. This finding was expected based on earlier studies (Lee & Williams 1974, 1975; Liu et al 1997).

As adults, the animals showed the predictable differences in behavioral and HPA responses to stress. On measures of plasma corticosterone responses to stress or behavioral fearfulness under conditions of novelty, the handled offspring of low LG-ABN mothers did not differ from either the handled or nonhandled offspring of high LG-ABN mothers. 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 or not they were handled 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 with the pedigree of their mothers. The answer was clear: The handled offspring of low LG-ABN mothers did not differ from the offspring of high LG-ABN mothers in their frequency of LG or ABN. As would be expected, the nonhandled offspring of low LG-ABN mothers were themselves low LG-ABN mothers. These findings provide further evidence for a nongenomic mechanism of inheritance.

The same was true of the effects on fearfulness. As adults, the offspring of the handled LG-ABN mothers, recipients of high levels of maternal LG, resembled the offspring of either handled or nonhandled, high LG-ABN mothers on measures of fearfulness. The offspring of nonhandled LG-ABN mothers, as would be expected, showed greater fearfulness in novel surroundings. Hence, the handling experience was transmitted to the next generation via the alteration in maternal behavior. The same pattern was observed for measures of CRF or glucocorticoid receptor gene expression: For hypothalamic CRF mRNA or hippocampal glucocorticoid receptor mRNA measures, adult offspring of handled LG-ABN mothers resembled the offspring of either handled or nonhandled, high LG-ABN mothers. Thus, it appears that individual differences in maternal behavior can be transmitted from one generation to the next through a behavioral mode of transmission. Moreover, the effects of an environmental event occurring in early life can also be transmitted into the next generation, and this effect is mediated by alterations in maternal behavior.

These findings suggest that environmental events can alter the trajectory of development not only in the affected offspring but also into the next generation. Almost 40 years ago, Denenberg (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 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 I have never understood, despite being published in *Nature*, the results of this remarkable study have remained almost ignored. The contribution of our laboratory (Francis et al 1999b) to this story is to have identified maternal behavior as a potential mediator for such transgenerational effects.

These findings are also consistent with those of recent studies on the potential effects of maternal behavior on the development of behavior and endocrine responses to stress in BALBc mice. BALBc mice are normally extremely fearful and show elevated HPA responses to stress. However, those cross-fostered to C57 mothers are significantly less fearful, with lower HPA responses to stress (Zaharia et al 1996). It is important that C57 mothers lick/groom their pups about twice as frequently as do BALBc mothers (Anisman et al 1998). Comparable findings have emerged with rat strains. Typically, Fisher 344 rats are more responsive to novelty and have increased HPA responses to acute stress compared with Long-Evans rats (Dhabhar et al 1993). Predictably, Long-Evans dams lick/groom their offspring significantly more often than do Fisher 344 mothers (Moore & Lux 1998). 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 excessively fearful animals. This is usually the reality of gene-environment interactions. The child of the depressed mother inherits not only the genetic vulnerability but also the depressed parent (e.g. Field 1998). This is also the reason why many epidemiological 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. The environment the parent provides commonly works in the same direction as the genetic influences; they are redundant forces. Knowledge of an animal's BALBc pedigree is sufficient to predict a high level of timidity in adulthood. Additional information on maternal care would add statistically little to the predictability—the two factors work in the same direction. But this is clearly different from concluding that maternal care is not relevant, and the results of the cross-fostering studies attest to the importance of such epigenetic influences. The misunderstanding on this point illustrates the degree to which the inappropriate use and interpretation of linear regression models to resolve the futile nature-nurture debate has served as a serious obstruction in developmental sciences.

MATERNAL CARE AND HIPPOCAMPAL DEVELOPMENT

In addition to the long-term effects described above, maternal care has immediate impact on endocrine function in infant rats. Tactile stimulation derived from mothers serves to dampen HPA activity in neonates, protecting the animals against the highly catabolic effects of adrenal glucocorticoids during a period of rapid development (see Levine 1994). Likewise, tactile stimulation from mothers stimulates the release of growth hormone (Schanberg et al 1984). Pups exposed to prolonged periods of maternal separation show increased levels of glucocorticoids and decreased levels of growth hormone. These effects can be reversed with “stroking” with a brush, a manipulation that mimics the tactile stimulation derived from maternal LG.

The results of these studies suggest that maternal LG can serve to promote an endocrine state that fosters growth and development. Variations in maternal care also appear to be related to individual differences in the synaptic development of selected neural systems that mediate cognitive development. As adults, the offspring of high LG-ABN mothers show enhanced spatial learning/memory in the Morris water maze (see Liu et al 2000) as well as in object recognition (Bredy et al 2000). The performance in both tasks is dependent on hippocampal function (e.g. Morris et al 1982, Squire 1992, Whishaw 1998, Wood et al 1999) and maternal care–altered hippocampal synaptogenesis. At either day 18 or day 90, there were significantly increased levels of neural cell adhesion molecule (N-CAM) or synaptophysin-like immunoreactivity on Western blot analyses in hippocampal samples from the high LG-ABN offspring, which suggests increased synapse formation/survival.

The influence of the hippocampus in spatial learning is thought to involve, in part at least, cholinergic innervation emerging from the medial septum (e.g. Quirion et al 1995). It is interesting that in microdialysis studies of adult offspring of high LG-ABN mothers, increased hippocampal choline acetyltransferase (ChAT) activity, acetylcholinesterase staining, and hippocampal basal and K⁺-stimulated acetylcholine release was found (Liu et al 2000). These findings suggest increased cholinergic synaptic number in the hippocampus of high LG-ABN offspring. There was also increased hippocampal levels of brain-derived neurotrophic factor (BDNF) mRNA in high LG-ABN offspring on day 8 of life (Liu et al 2000). BDNF is associated with the survival of cholinergic synapses in rat forebrain (Alderson et al 1990, Thoenen 1995, Friedman et al 1995). It is interesting that BDNF expression is enhanced by tactile stimulation in early development and decreased by maternal deprivation (Zhang et al 1997) and by elevated glucocorticoid levels (Chao et al 1998).

The expression of BDNF is regulated by N-methyl-D-aspartate (NMDA) receptor activation, and tactile stimulation has been shown to increase NMDA receptor expression in the barrel cells of mice (Jablonska et al 1996). There is increased mRNA expression of both the NR2A and NR2B subunits of the NMDA receptor in the offspring of high compared with low LG-ABN mothers at day 8 of age

(Liu et al 2000). These effects are associated with increased NMDA receptor binding. The results of a recent DNA array study (Diorio et al 2000) revealed the major class of effects on gene expression: (a) genes related to cellular metabolic activity (glucose transporter, cFOS, cytochrome oxydase, low-density-lipoprotein receptor, etc); (b) genes related to glutamate receptor function, including effects on the glycine receptor as well as those mentioned for the NMDA receptor subunits; and (c) genes encoding for growth factors, including BDNF, basic fibroblast growth factor (bFGF), and β -nerve growth factor. In each case, expression was greater than threefold higher in hippocampal samples from day-8 offspring of high LG-ABN mothers.

Naturally occurring variations in maternal LG and ABN were associated with the development of cholinergic innervation to the hippocampus, as well as with differences in the expression of NMDA receptor subunit mRNAs. In adults, there was increased hippocampal NR1 mRNA expression. These findings provide a mechanism for the differences observed in spatial learning and memory in adult animals. In adult rats, spatial learning and memory is dependent on hippocampal integrity; lesions of the hippocampus result in profound spatial learning impairments (e.g. Morris et al 1982, Squire 1992, Whishaw 1998, Wood et al 1999). Moreover, spatial learning is regulated by both cholinergic or NMDA receptor activation (e.g. Gage & Bjorkland 1986, Morris et al 1986, Quirion et al 1995) or NR1 subunit knockout animals (McHugh et al 1996). Likewise, hippocampal long-term potentiation, often considered a potential neural model for learning and memory (Bliss & Collingridge 1993, Bailey et al 1996), is enhanced by treatments that increase acetylcholine release (Calbresi et al 1998) or overexpression of NMDA receptor subunits at the level of the hippocampus (Tang et al 1999). Taken together, these findings suggest that maternal care increases hippocampal NMDA receptor levels, resulting in elevated BDNF expression, increased hippocampal synaptogenesis, and, thus, enhanced spatial learning in adulthood. These results are also consistent with the idea that maternal behavior actively stimulates hippocampal synaptogenesis in offspring through systems known to mediate experience-dependent neural development (e.g. Schatz 1990, Kirkwood et al 1993).

INDIVIDUAL DIFFERENCES IN MATERNAL BEHAVIOR

Variations in maternal behavior appear to have profound impact on neural development in rats. For these rodents, this may not be surprising. The first weeks of life do not hold a great deal of stimulus diversity for rat pups. Stability is the theme of the burrow, and the social environment in the first days of life is defined by the mother and the littermates. The mother, then, serves as a direct link between the environment and the developing animal. Thus, it seems reasonable that variations in mother-pup interaction would serve to carry so much importance for development. However, the parental mediation of environmental effects is not unique to the isolated confines of the rodent burrow. In humans, parental factors also serve

to mediate the effects of environmental adversity on development. For example, the effects of poverty on emotional and cognitive development are mediated by parental factors, to the extent that if such factors are controlled, there is no discernible effect of poverty on child development (Eisenberg & Earls 1975, Conger et al 1994, McLloyd 1998). These findings suggest that environmental adversity alters the behavior of the parents, which in turn affects the development of the child.

Human clinical research suggests that the social, emotional, and economic contexts 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, marital strife, and a lack of social and emotional support (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 (Berman 1990, Fairbanks 1996, Maestripieri 1999). But what are the neural mechanisms underlying such variations in maternal care, and how might they be regulated by environmental adversity?

The stress reactivity of offspring mirrors that of their mothers. Low LG-ABN mothers are more fearful than are high LG-ABN dams (Francis et al 2000). Likewise, their offspring are more fearful than are those of high LG-ABN mothers. The differences in fearfulness may, in fact, be a crucial point in understanding the neural basis for the individual differences in maternal behavior. Maternal behavior in rats emerges as a resolution of an interesting conflict (Rosenblatt 1994, Stern 1997, Fleming 1999). Unless they are in late pregnancy or lactating, female rats generally show an aversion to pups. Typical of the generally neophobic adult rat, it is the novelty of the pups that is the source of the aversion. Habituation to the novelty results in an altered set of responses. Thus, continuous exposure to the novel pups renders virgin females more likely to exhibit maternal behavior (i.e. pup sensitization) (Rosenblatt 1994; Bridges 1994, 1996). For maternally responsive females, the positive cues associated with pups are tactile, gustatory, and auditory (Stern 1997). Thus, pup stimuli can either be aversive, eliciting withdrawal, or positive, eliciting approach. The onset of maternal 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, increase maternal responsivity in nulliparous females (Fleming et al 1980). These findings suggest that the less-fearful female offspring of high LG-ABN mothers might be less averse to pups than the offspring of low LG-ABN mothers. This appears to be the case. Meaney & Champagne (2000) used the pup sensitization paradigm and found that the virgin female offspring of

high LG-ABN mothers became fully maternal in less than half the time that was required to induce a comparable effect in the offspring of low LG-ABN mothers. Moreover, simply screening adult female rats obtained from the breeder using the pup-sensitization paradigm showed that females that became maternal more readily (i.e. required a shorter period of exposure to pups) were subsequently high LG-ABN mothers.

Of course, even a primiparous female rat is fully maternal immediately after and even during parturition. For lactating females, pups are an intense source of attraction (e.g. Fleming 1999). The differences in the responses to pups of virgin vs lactating females are associated with the endocrine events of late pregnancy. Thus, a hormonal regimen that mimics the endocrine changes occurring in late pregnancy facilitates the expression of maternal behavior in virgin female rats (Bridges 1994, 1996; Rosenblatt 1994). It is interesting that the same treatment also reduces the animals' fear of novelty (Fleming et al 1989). One of the key components of these endocrine events is an estrogen surge that serves to induce oxytocin receptors in multiple reproductive tissues as well as in brain regions (Insel 1990, Pedersen 1995) that are known to mediate the expression of maternal behavior (Numan & Sheehan 1997). The increased sensitivity to oxytocin is associated with both a reduced level of fearfulness (McCarthy et al 1996, Windle et al 1997, Uvnas-Moberg 1997, Neumann et al 2000) and an increased maternal responsivity (Pedersen 1995). Among nonlactating females, there are no differences in oxytocin receptor levels except in the central n. of the amygdala, where receptor levels were higher in high LG-ABN mothers (Francis et al 2000). It is interesting that oxytocin appears to act at the central n. of the amygdala to reduce fearfulness (I Neumann, personal communication). Among lactating females, there were significantly higher levels of oxytocin receptors in the medial preoptic area, the bed n. of the stria terminalis, and the lateral septum in all animals (for a review, see Pederson 1995); however, the lactation-induced increase in receptors levels was substantially greater in high LG-ABN mothers (Francis et al 2000). Each of these brain regions has been implicated in the expression of maternal behavior in rats (Numan & Sheehan 1997). The results of a recent study (F Champagne, J Diorio, MJ Meaney, unpublished data) reflect the functional importance of such differences in oxytocin receptor levels. Thus, intracerebroventricular infusion of an oxytocin receptor antagonist on day 3 postpartum completely eliminated the differences in pup licking/grooming between high and low LG-ABN mothers.

Throughout most of pregnancy, progesterone levels are high and accompanied by moderate levels of estrogen. Then, prior to parturition, progesterone levels fall and there occurs a surge in estrogen levels. Both events are obligatory for the onset of maternal behavior. Estrogen appears to act at the level of the medial preoptic area to enhance the expression of maternal behavior (see Rosenblatt 1994). The influence of ovarian hormones on the onset of maternal behavior in rats appears to be mediated, in part, by effects on central oxytocinergic systems (Pederson 1995). Estrogen increases oxytocin receptor gene expression and receptor binding (e.g. de Kloet et al 1986, Johnson et al 1989, Bale et al 1995, Young et al 1997).

Intracerebroventricular administration of oxytocin rapidly stimulates maternal behavior in virgin rats (Pederson et al 1979, Fahrbach et al 1985), and the medial preoptic area appears to be a critical site. The effect of oxytocin is abolished by ovariectomy and reinstated with estrogen treatment. Moreover, treatment with oxytocin-antiserum or receptor antagonists blocks the effects of ovarian steroid treatments on maternal behavior (Pederson et al 1985, Fahrbach et al 1985). The mechanism underlying the differential effects of lactation on the induction of oxytocin receptors in high and low LG-ABN mothers appears to involve differences in estrogen sensitivity. Among ovariectomized females given estrogen replacement, there was a significantly greater estrogen effect on oxytocin receptor levels in the medial preoptic area in high compared with low LG-ABN animals (Champagne & Meaney 2000). The effect was apparent across a wide range of doses, and indeed there was little evidence for any effect of estrogen on oxytocin receptor levels in the medial preoptic area of low LG-ABN females. The fact that such differences occurred even in the nonlactating, ovariectomized state suggests the existence of stable differences in estrogen sensitivity in these animals. Although the mechanism for such differences in estrogen sensitivity is not yet clear, it is possible that these findings represent an active process of "feminization" such that the behavior of the mother toward her female offspring sensitizes selected brain regions to the effects of estrogen in adulthood and, thus, forms the basis for the transmission of individual differences in maternal behavior.

ENVIRONMENTAL REGULATION OF MATERNAL BEHAVIOR

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, etc, directly affect the emotional state of the mother and, thus, of maternal care. The effects of these environmental challenges on the development of the pups are then mediated by alterations in maternal care (see Figure 1). 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 (see Figure 1). Animals that are more fearful and anxious, such as low LG-ABN mothers, are more neophobic and lower in maternal responsiveness to pups than are the less-fearful animals. These effects could then serve as the basis for comparable patterns of maternal behavior in offspring (F1) and for the transmission of these traits to the subsequent generation (F2) (see Figure 1). These individual differences are transmitted to the offspring through 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 that serve to activate behavioral, endocrine, and autonomic responses to stress. Variations in maternal care in infant rats also influence the development

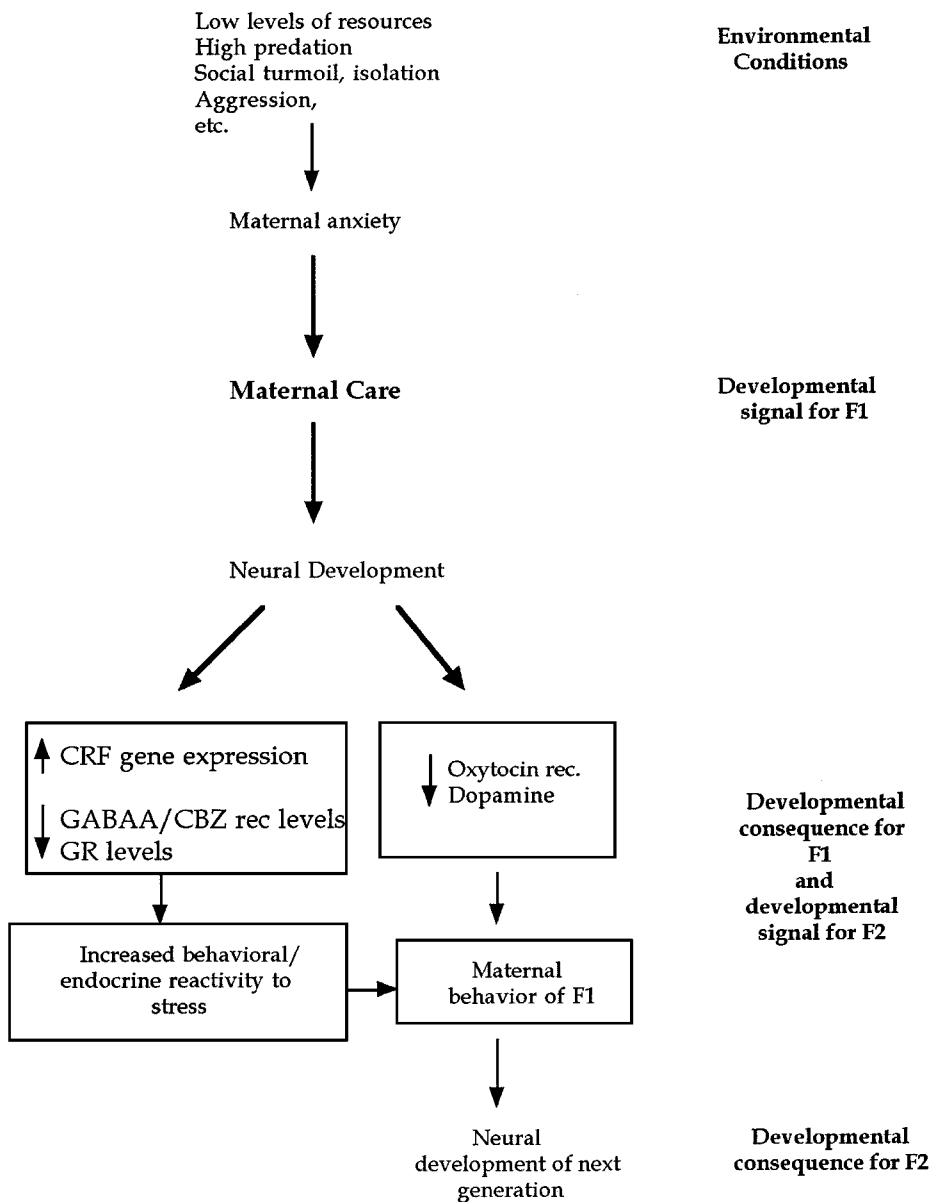


Figure 1 A schema representing the potential outcomes of the proposed relationship between environmental adversity and infant care. The key feature of this formulation is the hypothesized relationship between fearfulness and maternal behavior (for a review, see Fleming 1998). Thus, variations in maternal care affect the development of neural systems that mediate stress reactivity, which may then serve to influence maternal behavior. These effects then serve to influence the development of the subsequent generation and thus provide a basis for the transmission of individual differences in stress reactivity from one generation to the next. CRF, corticotropin-releasing factor; CBZ, central benzodiazepine; GR, glucorticoid receptor.

of neural systems, such as glucocorticoid and GABAA receptor systems, which provide an inhibitory tone over CRF synthesis and release.

Perhaps the most compelling evidence for this process emerges from the studies of Rosenblum, Coplan, and their colleagues (e.g. Coplan et al 1996, 1998). Bonnet macaque mother-infant dyads were maintained under one of three foraging conditions: low foraging demand, where food was readily available; high foraging demand, 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 these conditions were imposed, there were no differences in the nature of the mother-infant interactions. However, following a number of months of these conditions, there were highly significant differences. The VFD condition was clearly the most disruptive (Rosenblum & Andrews 1994). Mother-infant conflict increased in the VFD condition. Infants of mothers housed under these conditions were significantly more timid and fearful. Remarkably, these infants showed the signs of depression commonly observed in maternally separated macaque infants, even though 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 neural systems that mediate behavioral and endocrine responses to stress. As adults, monkeys reared under VFD conditions showed increased cerebrospinal fluid levels of CRF (Coplan et al 1996, 1998). 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 fascinating to see if these traits are transmitted to the next generation.

The critical issue here is the effect of environmental adversity on maternal behavior. In rats, females exposed to stress during pregnancy showed increased retrieval latencies (Fride et al 1985, Moore & Power 1986, Kinsley & Bridges 1988), a finding that would seem to reflect an effect of stress on maternal responsivity. In a recent study, Champagne & Meaney (2000) examined the effect of such gestational stress on maternal behavior in high and low LG-ABN mothers. Females previously defined as high or low LG-ABN mothers with their first litter were exposed either to restraint stress during the last half of gestation or to control conditions. We found that gestational stress decreased the frequency of maternal LG and ABN in high but not low LG-ABN mothers. Thus, a stressful environmental signal during gestation was sufficient to reverse completely the pattern of maternal behavior in high LG-ABN mothers. These findings led to us to question whether such effects of gestational stress would be apparent with a subsequent litter, even in the absence of any further stress. Indeed, the effects of gestational stress were fully evident with the third litter. The maternal behavior of high LG-ABN mothers exposed to gestational stress during an earlier pregnancy was indistinguishable from that of low LG-ABN mothers. The results raise what, in our minds, is a fascinating possibility: Chronic stress during gestation results in a sustained alteration in one

or more neural systems that mediate the expression of maternal behavior, resulting in long-term changes in maternal behavior.

Summary

Taken together, these findings suggest that environmental adversity alters the emotional well-being of the mother: Chronic stress increases anxiety and fearfulness and, thus, decreases maternal responsiveness, which in turn influences the development of stress reactivity in the offspring. For humans, these are not isolated conditions: One in five teens and one in six adult women experience abuse during pregnancy (Newberger et al 1992, Parker et al 1994). Also, in humans, Fleming (1988) reported that many factors contribute to the quality of the mother's attitude toward 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 toward their babies (also Field 1998). Moreover, there is evidence for the behavioral transmission of anxiety. Highly anxious mothers are more likely to have children who are shy and timid, and the behavior of the mother predicts the level of such behavioral inhibition in the child (Hershfeld et al 1997a,b). It was recently found that scores on parental bonding measures were correlated with autonomic, HPA and mesolimbic dopamine responses to stress (Preussner et al 2000): Young adults who described cold, distant relationships with their parents showed increased glucocorticoid and cardiovascular responses to stress, as well as evidence for increased dopamine release in the ventral striatum. More extreme variations in parental care have predictable results. Heim et al (2000) recently reported that, as adults, victims of abuse in early life show increased endocrine and autonomic responses to stress.

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 & King 1987): Englishmen inherit England, as Francis Galton remarked. We believe that the findings on intergenerational transmission via maternal behavior represent an adaptive approach to development. Under conditions of increased environmental demand, it is commonly in an animal's interest to enhance its behavioral (e.g. vigilance, fearfulness) and endocrine (HPA and metabolic/cardiovascular) responsiveness to stress (see above). These responses promote detection of potential threat, avoidance learning, and metabolic/cardiovascular responses that are essential under the increased demands of the stressor. Because offspring usually inhabit niches similar to those of their parents, the transmission of these traits from parent to offspring could serve to be adaptive. A metaphor for this argument exists in the physiology of the thrifty phenotype in rodents (Neel 1962, Hales & Barker 1992). In response to the deprivation of energy substrates in fetal life, rodents show a

pattern of development that favors energy conservation and an increased capacity for both gluconeogenesis and lipolysis in adulthood. Both effects appear to reflect “anticipatory” patterns of development that would be adaptive under repeated periods of food shortages. It is interesting that these effects are mediated by sustained changes in the expression of genes in hepatic tissues that mediate glucose and fat metabolism (Desai et al 1995, Bauer et al 1998, Phillips 1998, Seckl et al 1999). I believe that the effects of maternal care of the expression of genes involved in the regulation of behavioral and endocrine responses to stress reflect a comparable effect.

The key issue here is that of the potential adaptive advantage of the increased level of stress reactivity apparent in the offspring of low LG-ABN mothers. This point was addressed earlier in relation to the handling studies, and the potential advantages of increased HPA responsiveness to stress in the nonhandled animals. In the current context, the research of Farrington et al (1988) and Haapasap & Tremblay (1994), for example, on young males growing-up in low socio-economic status (SES) and high crime environments provides an excellent illustration of the potential advantages of increased stress reactivity. In this environment, the males who were most successful in avoiding the pitfalls associated with such a “criminogenic” environment were those who were shy and somewhat timid. Under such conditions, a parental rearing style that favored the development of a greater level of stress reactivity to threat would be adaptive. It is thus perhaps understandable that parents occupying a highly demanding environment would transmit to their young an enhanced level of stress reactivity in “anticipation” of a high level of environmental adversity. Such a pessimistic developmental profile (see Figure 1) would be characterized (*a*) by an increased level of hypothalamic and amygdaloid CRF gene expression and (*b*) in patterns of gene expression that dampen the capacity of inhibitory systems, such as the GABAA/CBZ receptor complex and the hippocampal glucocorticoid receptor system. In addition, a pessimistic developmental profile provides less investment in metabolically expensive synaptic systems, such as the hippocampal circuits (see also Sapolsky 1992). This might also be adaptive because elevated glucocorticoid levels associated with environmental adversity and increased stress reactivity would serve to damage hippocampal systems (see Meaney et al 1988, Sapolsky 1992). In contrast, more favorable environments would encourage an optimistic pattern of development, characterized by more modest levels of stress reactivity and increased hippocampal synaptogenesis. The quality of the environment influences the behavior of the parent, which in turn is the critical factor in determining whether development proceeds along an optimistic or a pessimistic pattern of development. The obvious conclusion is that there is no single ideal form of parenting: Various levels of environmental demand require different traits in offspring.

A final issue here concerns the cost of such increased stress reactivity. A shy and timid child in an urban slum may be at an advantage with respect to the demands of a menacing environment. The question is whether such traits would later also confer an increased risk for stress-induced illness. I 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 socioeconomic environment, in early life, a process mediated by effects of adversity on parental care.

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