Variations in Maternal Care in Infancy Regulate the Development of Stress Reactivity

Christian Caldji, Josie Diorio, 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/grooming on the development of central systems that serve to activate (corticotropin-releasing factor) or inhibit (γ-aminobutyric acid) the expression of behavioral and endocrine responses to stress through effects on forebrain noradrenergic systems. Importantly, individual differences in maternal care are transmitted from mother to daughter, providing a mechanism for the behavioral transmission of individual differences in stress reactivity across generations.


Key Words: Maternal care, stress reactivity, GABA_A receptor, corticotropin-releasing factor

Introduction

Stress is a risk factor for a variety of illnesses, ranging from metabolic and cardiovascular disorders to mental illness. The pathways by which stressful events can promote the development of such divergent forms of illness involve the same hormones that ensure survival during a period of stress (Brindley and Rolland 1989; Dallman et al 1995; De Kloet et al 1998; McEwen 1998; McEwen and Stellar 1993; Munck et al 1984). Stress-induced increases in sympathoadrenal release of catecholamines, adrenaline and noradrenaline, and adrenal glucocorticoids orchestrate a move to catabolism, increasing lipolysis and mobilizing glucose reserves. These actions serve to increase the availability and distribution of energy substrates. There are also emotional and cognitive responses to stressors that are mediated by adrenal hormones as well as the activation of central corticotropin-releasing factor (CRF) systems (Bakshi et al 2000; Nemeroff 1996). During stress, feelings of apprehension and fear predominate; individuals become hypervigilant. The level of attention directed to the surrounding environment is increased at the expense of the ability to concentrate on tasks not related to the stressor (e.g., Arnsten 1998). Glucocorticoids act on brain structures such as the hippocampus and amygdala to disrupt episodic memory (Lupien et al 1998). At the same time, glucocorticoids and catecholamines act on areas of the brain, such as the amygdala, to enhance learning and memory for emotionally relevant stimuli (e.g., Cahill and McGaugh 1998; Davis et al 1997; Pitkanen et al 1998; Quirarte et al 1997). Although these responses are highly adaptive, chronic activation of these systems can promote the development of hyperlipidemia, hypertension, chronic immunosuppression and decreased viral resistance, states of anxiety and dysphoria, sleep disorders, etc. (Chrousos and Gold 1992; McEwen 1998; Nemeroff 1996; Rosmond et al 1998).

Despite the profound risk associated with chronic stress, it is clear that measures of life events take us only so far in understanding why and when certain individuals develop chronic illness. Simply put, under comparable levels of environmental demand, some individuals fall ill and others do not. Recent findings on posttraumatic stress disorder (PTSD) underscore this point. There is considerable variation in the rates of PTSD associated with such obvious trauma as violent assault (e.g., Breslau et al 1998) and evidence for high-risk populations (Yehuda et al 1998). These findings suggest the existence of a predisposition such that, when stress meets vulnerability, disease is a more likely outcome. The next and obvious question concerns the origin and nature of this predisposition.

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; McCauley et al 1997). 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 Robins 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. And the risk is not unique to mental health. Russak and Schwartz (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 fourfold greater risk of chronic illness, including not only depression and alcoholism, but also heart disease and type II diabetes.

Thus, individual differences in parental care are related to the health of the offspring. We have argued that this effect is, in part at least, mediated by parental influences on the development of neural systems that underlie the expression of behavioral and endocrine responses to stress (Coplan et al 1996; DeBellis et al 1994; Francis and Meaney 1999; Helm et al 1977; Higley et al 1991; Kraemer et al 1989; Meaney et al 1996; Seckl and Meaney 1994). Parental rearing that results in enhanced reactivity to stress appears to increase the risk for illness in later life (e.g., Heim et al 1977). 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 forms of chronic illness (see above and Chrousos and Gold 1992; McEwen 1998; Owens and Nemeroiff 1994; Sapolsky 1994). Although the stress hormones have emerged as reasonable mediators of the relationship between stress and disease, the critical question here concerns the nature of the underlying predisposition and its relationship to early life events.

**Maternal Care and the Development of Individual Differences in Stress Reactivity**

We have attempted to address this issue with studies on the role of maternal care in neural development in the rat. Interestingly, there are substantial, naturally occurring variations in maternal licking/grooming in rat dams. Maternal licking/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 ($r = +.91; 30$) across mothers. The differences in maternal behavior in the high- and low-licking/grooming arched-back nursing mothers are not unique to the first litter (Francis et al 1999b). Across a group of 22 dams we found a correlation of $+.87$ between the licking/grooming of the first and second litters. These findings are comparable to those of primate studies in which individual differences in maternal behavior remained consistent across infants (e.g., Fairbanks 1996; Maestripieri 1999). In one series of studies, mothers were divided into two groups: high- or low-licking/grooming arched-back nursing. Note there were no differences between these groups in the overall amount of time in contact with pups (Caldji et al 1998; Liu et al 1997). We then allowed the offspring to grow to adulthood and examined hypothalamic–pituitary–adrenal (HPA) and behavioral responses to stress (Liu et al 1997). As adults, the offspring of high-licking/grooming arched-back nursing mothers showed reduced plasma adrenocorticotropic hormone and corticosterone responses to restraint stress. The offspring of the high-licking/grooming arched-back nursing mothers showed a more modest HPA response to stress. In the adult rat, glucocorticoids act at specific receptor sites within selected brain regions, such as the hippocampus, to decrease CRF synthesis in paraventricular nucleus of the hypothalamus (PVNh) neurons (De Kloet et al 1998; Jacobson and Sapolsky 1991). The high-licking/grooming arched-back nursing animals also showed significantly increased hippocampal glucocorticoid receptor messenger RNA (mRNA) expression, enhanced glucocorticoid negative feedback sensitivity, and decreased hypothalamic CRF mRNA levels. Moreover, the magnitude of the corticosterone response to acute stress was significantly correlated with the frequency of both maternal licking/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 CRF mRNA expression (all $r > .70$).

The offspring of the high- and low-licking/grooming arched-back nursing mothers also differed in behavioral responses to novelty (Caldji et al 1998; Francis et al 1999b). As adults, the offspring of the low-licking/grooming arched-back nursing rats showed increased startle responses, decreased open field exploration, and longer latencies to eat food provided in a novel environment, reflecting a greater fear of novelty. These findings are consistent with the results of the classic studies of Levine and Denenberg (Denenberg et al 1963; Hess et al 1969; Levine 1957, 1962; Levine et al 1967) showing that perhaps the greatest effect of early environmental events is on the development of responses to conditions of threat (implied danger).

The offspring of the low-licking/grooming arched-back nursing mothers show increased CRF receptor levels in the locus coeruleus and decreased central benzodiazepine receptor levels in the basolateral and central nuclei of the amygdala as well as in the locus coeruleus and increased CRF mRNA expression in the central nucleus of the amygdala (D. Francis et al, unpublished data). Corticotropin-releasing factor neurons from the central nucleus of the amygdala project to noradrenergic cell body regions such as the locus coeruleus, where CRF serves to activate
neuronal firing and noradrenaline release (Gray and Bingaman 1996; Koegler-Muly et al 1993; Lavicky and Dunn 1993; Valentino et al., 1998) and behavioral states of fear (e.g., Butler et al 1990; Liang et al 1992). Predictably, stress-induced increases in PVN 1 levels of noradrenaline were significantly higher in the offspring of the low-licking/grooming arched-back nursing offspring (Caldji et al 1999; Liu et al 2000; for a recent review on the CRF–noradrenaline systems in these animals, see Francis et al 1999a).

**Maternal Care and the γ-Aminobutyric Acid (GABA) A Receptor Complex**

The effects of maternal care are also apparent on systems that normally serve to inhibit emotional, behavioral, and endocrine responses to stress. The most potent source of such inhibitory regulation is the GABAergic system. In recent studies we have described the impressive effects of variations in maternal care on the development of the forebrain GABAergic system. One interesting feature of these studies is the regional specificity of these effects, which prominently feature the amygdala and the locus coeruleus.

The GABA A receptor complex, which includes a benzodiazepine-binding site, is comprised of multiple subunits most commonly arranged in a pentameric structure (for a recent review, see Mehta and Ticku 1999). The GABA A receptor complex is comprised of α (at least six variants), β (at least three variants), and γ (at least three variants) subunits, with more recently identified δ, ε, ρ, and π subunits. In the rat forebrain the native GABA A receptor complexes most often take the form of two α, two β, and one γ subunit or of two α, one β, and two γ subunits. It is thought that the interface between the α and γ subunits forms the benzodiazepine receptor site. Thus, receptor complexes with two α and two γ subunits would have two benzodiazepine-binding sites (e.g., Khan et al 1996). Subunit composition can be critical. The α 4 subunit does not appear to participate in the formation of a functional benzodiazepine receptor site. Likewise the γ 3 subunit does not confer benzodiazepine binding. In addition, the α subunits confer varying levels of affinity for GABA upon the GABA A receptor.

In earlier studies (Caldji et al 2000) we found that repeated periods of prolonged maternal separation resulted in decreased GABA A receptor binding, as well as diminished central (type I) benzodiazepine receptor levels. These effects are associated with alterations in some of the GABA A receptor subunits noted above. Although maternal separation studies provide an interesting insight into the potential role of mother–infant interactions in development, prolonged states of deprivation are highly disruptive to normal physiology. The results of more recent studies have focused on the offspring of high- and low-licking/grooming arched-back nursing mothers.

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The results are summarized from experiments comparing the adult offspring of high- and low-licking/grooming arched-back nursing mothers.

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Table 1. A Summary of Differences in γ-Aminobutyric Acid A Receptor Subunit Messenger RNA Expression across Multiple Brain Regions

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A series of situ hybridization studies have offered insight into 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 GABA A receptor complex in the offspring. These findings are summarized in Table 1. The most obvious differences in relation to the receptor binding profile lie in the levels of the mRNAs for the γ 1 and γ 2 subunits. Both subunits actively contribute to the formation of a functional central benzodiazepine-binding site. Note that the γ 1 and γ 2 mRNA levels are increased in the amygdala and the locus coeruleus of the adult offspring of high-licking/grooming arched-back nursing mothers. There are no differences in the mRNA levels for the γ 3 subunit, which does not appear to contribute to a functional central benzodiazepine-binding site. Note that we have not, as yet, examined the expression of the short and long variants of the γ 2 subunit. These findings provide a
mechanism for the increased central benzodiazepine receptor binding in the high-licking/grooming arched-back nursing offspring. But such differences are not unique to the γ subunits.

Levels of α1 mRNA are significantly higher in the amygdala and locus coeruleus of high-licking/grooming arched-back nursing offspring. Similar differences are also apparent in the medial prefrontal cortex. The α1 subunit appears to provide the most efficient form of the GABA_A receptor complex, through increased receptor affinity for GABA. The γ6 subunit appears largely confined to the cerebellum. However, there are effects of maternal care on the expression of the remaining α subunits and this is where, in our minds, the story becomes most intriguing.

After viewing the α1 subunit data it was tempting for us to ascribe the decreased GABA_A receptor binding simply to a decreased level of subunit expression. This is not the case. The adult offspring of the low-licking/grooming arched-back nursing mothers actually show increased expression of the mRNAs for the α3 and α4 subunits in the amygdala and the locus coeruleus. Two features of these subunits are noteworthy. First, they confer upon the GABA_A receptor complex a reduced affinity for GABA, relative to the α1 subunit. Second, the α4 subunit does not contribute to the formation of a central benzodiazepine receptor site.

These differences in GABA_A receptor subunit expression are also reflected in the central benzodiazepine receptor binding. Interestingly, though the α3 subunit contributes to the formation of a benzodiazepine receptor binding site, those sites are of the type II rather than type I variety. [3H]Zolpidem can be used to distinguish type I and type II benzodiazepine receptor sites, since this radioligand has little affinity for the type II receptor (e.g., Arbilla et al 1986). The previously reported differences in benzodiazepine receptor binding capacity between the adult offspring of high- and low-licking/grooming arched-back nursing 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 α1 subunit alone produces a type I benzodiazepine receptor site. GABA_A receptor complexes containing α3 or α5 subunits display type II benzodiazepine receptor pharmacology, although the α5-containing receptors lack high-affinity zolpidem binding (type I benzodiazepine receptor; e.g., Hadingham et al 1993). The α4 subunit, as mentioned above, does not produce a benzodiazepine receptor type of either variety (e.g., Khan et al 1996).

In conclusion, the differences in type I benzodiazepine receptor binding in the adult offspring of the high- and low-licking/grooming arched-back nursing mothers appear to emerge from tissue-specific differences in GABA_A receptor complex subunit composition. In the offspring of high-licking/grooming arched-back nursing mothers there is increased expression of the α1, γ1, and γ2 subunits, all of which contribute to the formation of GABA_A receptor complexes with type I benzodiazepine receptor binding. In contrast, there is increased expression of the α3 and α4 subunits in the offspring of the low-licking/grooming arched-back nursing mothers. Both subunits result in reduced affinity for GABA and neither of these subunits contributes to type I benzodiazepine receptor variants. These subunit profiles in both groups actively contribute to the differences in type I benzodiazepine receptor binding observed in the offspring of the high- and low-licking/grooming arched-back nursing mothers. This is not simply the case of a deficit in subunit expression in the offspring of the low-licking/grooming arched-back nursing mothers, but of an apparently active attempt to maintain a specific benzodiazepine receptor profile in selected brain regions.

It is important to note that, to date, such differences have been described only at the level of mRNA. We are currently in the midst of studies, including those using immunoprecipitation of native receptors, that would confirm that differences in gene expression are, in fact, associated with differences in GABA_A receptor composition.

Another critical question concerns the link between these differences in GABA_A/benzodiazepine receptor binding and the functional differences in stress reactivity that we observe between the offspring of high- and low-licking/grooming arched-back nursing mothers. Results of knockout/knockdown models as well as pharmacologic interventions suggest that such differences might indeed be associated with underlying differences in stress reactivity and, perhaps, with a vulnerability to stress-related anxiety disorders. This idea is certainly consistent with a wealth of studies on GABA_A/benzodiazepine receptor pharmacology. Benzodiazepine agonists have been thought to exert anxiolytic effects via their actions at a number of limbic areas (e.g., Gonzalez et al 1996; Gray 1987; Persold and Treit 1995; Thomas et al 1985). To date, the evidence is perhaps strongest for benzodiazepine effects at the level of the lateral and central nuclei of the amygdala. Thus, the direct administration of drugs that enhance GABAAergic activity via actions at benzodiazepine receptor sites into the amygdala yields an anxiolytic effect (Hodges et al 1987). There is evidence for benzodiazepine action directly at the level of both the lateral and
the central nuclei of the amygdala. The results of recent studies have indicated that the CRF neurons within the amygdala might serve as a specific target for benzodiazepine effects. The benzodiazepine receptor agonist alprazolam has been shown to reduce CRF content in the locus coeruleus (Owens et al 1991). The amygdala is a significant source of the CRF in the region of the locus coeruleus (Gray et al 1989; Koegler-Muly et al 1993; van Bockstaele et al 1996). de Boer et al (1992) have shown that benzodiazepine administration attenuates the effects of intracerebroventricular CRF treatment, suggesting that the CRF system is a target for the anxiolytic effects of the benzodiazepines (also see Owens et al 1991; Swerdlow et al 1986). We have recently reported significantly elevated CRF mRNA in amygdalae of MS and, to a lesser extent, NH rats, as compared with H rats (P.M. Plotsky et al, unpublished observations). The same pattern was observed for CRF immunoreactivity in the locus coeruleus (also see Ladd et al 1996). Considering the importance of this amygdaloid CRF system in mediating behavioral responses to novelty (Hitchock and Davis 1986; Krahm et al 1988; Liang et al 1992), we propose that benzodiazepine–CRF interactions within the amygdala serve as a critical neural substrate for the behavioral differences in response to novelty observed among H, NH, and MS rats.

In addition to the amygdala, there is also evidence for the importance of benzodiazepine action at the level of the frontal cortex (Lippa et al 1979) for anxiolytic effects. It appears likely that a variety of structures are involved in mediating the anxiolytic effects of benzodiazepines depending upon the nature of the stressor (Gonzalez et al 1996). In this respect, since we have found alterations at several sites, the differences in GABAceptors of central benzodiazepine receptor binding as a function of early rearing condition could serve to influence a wide range of behavioral and endocrine responses.

These findings are also interesting in light of the substantial differences in HPA responses to stress in the adult offspring of high- and low-licking/grooming arched-back nursing mothers (Liu et al 1997). Hypothalamic–pituitary–adrenal responses to stress are mediated by the release of CRF from the PVN. Interestingly, we found no differences in GABAceptors of central benzodiazepine receptor binding in the PVN. However, there were highly significant differences in the noradrenergic cell body regions, including the locus coeruleus and the nucleus tractus solitarius. Noradrenergic input to the PVN provides the major known source of activation for CRF synthesis and release (Pacak et al 1995; Plotsky 1991; Plotsky et al 1989). Thus, the increased GABAceptors of central benzodiazepine receptor binding observed in these regions in the high-licking/grooming arched-back nursing offspring may also serve to dampen HPA responses to stress by decreasing the magnitude of the noradrenergic signal. Precisely the same logic can be applied in consideration of the differences in behavioral responses to stress, considering the importance of the CRF–noradrenaline system in the expression of fear (Bakshi et al 2000; Dunn and Berridge 1990; Koob et al 1994).

Perhaps the most interesting data on this point emerge from human studies of benzodiazepine type I receptors. Malizia et al (1995) reported a significant decrease in forebrain type I benzodiazepine receptor levels using positron emission tomography with unmedicated patients with a history of panic disorder. Although these studies are preliminary, the findings are certainly consistent with those using pharmacologic measures of benzodiazepine receptor sensitivity. Glue et al (1995) found that subjects who were high on measures of neuroticism showed reduced sensitivity to the benzodiazepine midazolam. In a series of studies Roy-Byrne and colleagues (1990, 1996) found reduced sensitivity to diazepam in patients with panic disorders and obsessive–compulsive disorders and proposed that the reduced central benzodiazepine sensitivity was related to the phenomenon of anxiety. Although these studies have not directly shown decreased central benzodiazepine receptor levels, the findings are certainly consistent with the idea that decreased receptor levels in humans could be related to increased vulnerability to anxiety disorders. The hypothesis here is that there exists an endogenous anxiolytic ligand for the central benzodiazepine receptor and that decreased central benzodiazepine-binding sites would thus result in enhanced fearfulness in the face of threat. The results of at least one study directly relate the effects of rearing condition on central benzodiazepine receptor levels with those on behavior. Escorihuela et al (1992) found that postnatal handling improved performance in a test of a two-way avoidance task. The effect of handling was significantly reversed in animals treated acutely with the central benzodiazepine receptor antagonist RO 15-1788.

Although the relevant source of GABAergic inhibition is not yet defined, one likely candidate lies in the medial prefrontal cortex, which has extensive projections to the amygdala as well as to the locus coeruleus and the nucleus tractus solitarius (e.g., Van der Kooy et al 1984). We found that expression of glutamic acid decarboxylase (GAD) mRNA, the rate-limiting enzyme in GABA synthesis, was significantly increased in the medial prefrontal cortex neurons of the adult offspring of high-licking/ grooming arched-back nursing mothers relative to offspring of low-licking/grooming arched-back nursing mothers. Considering the uncertainty regarding relevant projections as well as the relationship between GAD gene expression and actual GABA signaling, these findings are
certainly very preliminary. Nevertheless, the differences in GAD expression suggest the possibility of differential GABA inhibition in the offspring of high- and lowlicking/grooming arched-back nursing mothers.

**Transmission of Individual Differences in Stress Reactivity**

Such effects of maternal behavior suggest that variations in maternal care might serve as a possible mechanism by which selected traits might be transmitted from one generation to the next. Interestingly, individual differences in maternal behavior show intergenerational transmission. The female offspring of high-licking/grooming arched-back nursing mothers showed significantly more licking/grooming and arched-back nursing than did the female offspring of low-licking/grooming arched-back nursing mothers. 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 1999). There is also evidence for transmission of individual differences in parental styles falling within the normal range. Fairbanks (1989, 1996) found that daughters who were reared by mothers who 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 mothers rejecting their infants was correlated with the rejection rate of the mothers’ mothers. In primates, such individual differences in maternal behavior may be revealed in juvenile, nulliparous females. In all cases these findings were independent of the social rank of the mother. In humans, 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 perhaps common process of intergenerational transmission of maternal behavior. The critical question here concerns the mechanism underlying this intergenerational transmission of individual differences in behavior.

We (Francis et al 1999b) have provided evidence for a nongenomic transmission of maternal behavior. In one study, we performed reciprocal cross-fostering of the offspring of low- and high-licking/grooming arched-back nursing mothers. The primary concern here was that the wholesale fostering of litters between mothers is known to affect maternal behavior (Maccari et al 1995). To avert this problem and maintain the original character of the host litter, no more than two of 12 pups were fostered into or from any one litter. The critical groups of interest are the biological offspring of low-licking/grooming arched-back nursing mothers fostered onto high-licking/grooming arched-back nursing dams, and vice versa. The control groups included 1) the offspring of low-licking/grooming arched-back nursing mothers fostered onto other low-licking/grooming arched-back nursing mothers as well as offspring of high-licking/grooming arched-back nursing dams fostered onto other high-licking/grooming arched-back nursing mothers, 2) sham-adoption animals that were simply removed from the nest and fostered back to their biological mothers, and 3) unmanipulated pups. The limited cross-fostering design did not result in any effect on group differences in maternal behavior. Hence, the frequency of pup licking/grooming and arched-back nursing across all groups of high-licking/grooming arched-back nursing mothers was significantly higher than that for any of the low-licking/grooming arched-back nursing dams, regardless of litter composition.

The results of the behavioral studies are consistent with the idea that the variations in maternal care are causally related to individual differences in the behavior of the offspring. The biological offspring of low-licking/grooming arched-back nursing dams reared by high-licking/grooming arched-back nursing mothers were significantly less fearful under conditions of novelty than were any of the offspring reared by low-licking/grooming arched-back nursing mothers, including the biological offspring of high-licking/grooming arched-back nursing 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 for fearfulness. As adults, the female offspring of low-licking/grooming arched-back nursing dams reared by high-licking/grooming arched-back nursing mothers did not differ from normal, high-licking/grooming arched-back nursing offspring in the frequency of pup licking/grooming or arched-back nursing. The frequency of licking/grooming and arched-back nursing in animals reared by high-licking/grooming arched-back nursing mothers was significantly higher than in any of the low-licking/grooming arched-back nursing groups, and again this included female pups originally born to high-licking/grooming arched-back nursing mothers but reared by low-licking/grooming arched-back nursing dams. Individual differences in fearfulness or maternal behavior mapped onto those of the rearing mother, rather than the biological mother.

A second series of studies was designed to map onto early family intervention programs. Handling increases maternal licking/grooming and arched-back nursing. Handling pups, in fact, turns low-licking/grooming arched-back nursing dams into high-licking/grooming arched-back nursing mothers (Francis et al 1999b; Liu et al 1997).
As adults, the handled F2 offspring of such mothers resemble the offspring of high-licking/grooming arched-back nursing mothers, a finding that is consistent with the nongenomic transmission hypothesis. We then studied the F3 generation, focusing on the offspring of the handled and nonhandled F2 offspring of low-licking/grooming arched-back nursing mothers. Bear in mind, we refer to these mothers as low-licking/grooming arched-back nursing because they are derived from low-licking/grooming arched-back nursing mothers themselves. The low-licking/grooming arched-back nursing mothers with handled pups behave in a manner that is indistinguishable from high-licking/grooming arched-back nursing dams, as do their female offspring. We (Francis et al 1999b) found that the handled offspring of low-licking/grooming arched-back nursing mothers resemble the offspring of high-licking/grooming arched-back nursing dams on measures of behavioral and endocrine responses to stress, hypothalamic CRF, and hippocampal glucocorticoid receptor mRNA expression, as well as central benzodiazepine receptor binding. These findings suggest individual differences in gene expression can also be transmitted across generations via a nongenomic mechanism. Moreover, an environmental manipulation that alters maternal behavior can then serve to affect neural development in subsequent generations through the transmission of these alterations in parental care.

These findings are consistent with the results of studies 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. Although the selective breeding suggests a genetic background, the expression of the hypertensive trait is also influenced by epigenetic factors (McCarty et al 1992). Spontaneously hypertensive rat pups reared by wild-type, WKY mothers do exhibit hypertension to the extent of kin reared by SHR dams. When borderline hypertensive rats, a hybrid formed by SHR–WKY matings, are reared by WKY mothers, they do not express the spontaneous hypertensive phenotype.

The potential effects of maternal behavior on the development of behavior and endocrine responses to stress in BALBc mice have been examined. The BALBc strain mice are normally very fearful and show 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 normally lick and groom their pups about twice as frequently as 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 relative to Long–Evans rats. Moore and Lux (1998) reported that Long–Evans dams lick/groom their offspring significantly more often than do Fisher 344 mothers.

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 development. Because parents provide both genes and environment for their biological offspring, the offspring’s environment is therefore, in part, correlated with their genes (e.g., West and King 1987). 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.

The value of this process is that it can provide for variation. If the genetically determined trajectory is not adaptive for the animal, then development moving in the direction of the current environmental signal would be of adaptive value. Hence, environmental events can alter the path of the genetically established trajectory in favor of more adaptive outcomes. This, of course, is the adaptive value of plasticity and the essence of epigenesis.

To our thinking, these are adaptive processes. We believe that the transmission of individual differences in stress reactivity from mother to offspring can provide an adaptive level of “preparedness” for the offspring. Under conditions of increased environmental demand, it is commonly in the animal’s interest to enhance behavioral (e.g., vigilance, fearfulness) and endocrine (HPA and metabolic/cardiovascular) responsivity to stress. These responses promote detection of potential threats, avoidance learning, and the mobilization of energy reserves that are essential under the increased demands of the stressor. Since the offspring usually inhabit a niche that is similar to that of their parents, the transmission of these traits from parent to offspring could serve to be adaptive. In this context it is understandable that parents inhabiting a very demanding environmental niche might “transmit” a high level of stress reactivity to their offspring. The research of Farrington et al (1988) and Tremblay (e.g., Haapasap and Tremblay 1994) on young males growing up in low–socioeconomic status and high-crime urban environments provides an excellent illustration of the potential advan-
tages of increased stress reactivity. In this environment, the males that 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 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 here concerns the cost of such increased stress reactivity. The shy and timid child in the urban 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 an increased risk for stress-induced illness. We argue that they would and that this risk reflects the cost of adaptation to a high level of environmental demand, such as a low–socioeconomic status environment, in early life. This level of plasticity also suggests a certain degree of reversibility.

Conclusions

It may be surprising 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 serves as a primary link between the environment and the developing animal (Levine 1975). It seems reasonable that variations in mother–pup interaction would serve to have so much importance in development. Indeed, it appears that the mother serves to transduce information pertaining to the environment to her offspring. It may be surprising that rather subtle variations in parental care are statistically neutralized, there is no significant effect of socioeconomic status on child development (Conger et al 1994; Eisenberg and Earls 1975; Mcloyd 1998). Hence, environmental effects on neural development during infancy, including systems that regulate stress reactivity in later life, may be commonly mediated through alterations in parental care.

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