Maternal programming of defensive responses through sustained effects on gene expression

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There are profound maternal effects on individual differences in defensive responses in species ranging from plants to insects to birds. In this paper, we review data from the rat that suggest comparable forms of maternal effects on defensive responses to stress, which are mediated by the effects of variations in maternal behaviour on gene expression. Under conditions of environmental adversity, maternal effects enhance the capacity for defensive responses in the offspring. These effects appear to "program" emotional, cognitive and endocrine systems toward increased sensitivity to adversity. In environments with an increased level of adversity, such effects can be considered adaptive, enhancing the capacity for responses that have immediate adaptive value; the cost is an increased risk for multiple forms of pathology in later life.

Il y a de profonds effets maternels sur les différences individuelles au niveau des réactions défensives chez des espèces allant des végétaux aux insectes et aux oiseaux. Dans cet article, nous étudions des données sur le rat qui indiquent des formes comparables d'effets maternels sur les réactions défensives face au stress, dont les facteurs médiateurs sont les effets des variations du comportement de la mère sur l'expression génique. Dans des conditions d'adversité environnementale, les effets maternels améliorent la capacité de réaction défensive chez les rejetons. Ces effets semblent «programmer» les systèmes affectif, cognitif et endocrinien pour les rendre plus sensibles à l'adversité. Dans des environnements où l'adversité est plus importante, on peut considérer que ces effets sont adaptatifs et améliorent la capacité de produire des réactions ayant une valeur adaptative immédiate, ce qui se fait au prix d'un risque accru de multiples formes de pathologies plus tard dans la vie.

Introduction

The quality of family life influences the development of individual differences in vulnerability to illness throughout life.[1] Such effects include vulnerability for obesity, metabolic disorders and heart disease as well as affective disorders and drug abuse.[2–4] Recent findings from epidemiological studies[5–9] as well as from primate models[10] suggest that developmentally determined vulnerability emerges from the interaction between genotype and early environmental events, including early life adversity. Critical questions concern the identity of the relevant genomic targets, the nature of the gene–environment interactions and their relation to phenotype.

Stress diathesis models are proposed as explanations for the effects of early life on health in adulthood and suggest that adversity in early life alters the development of neural systems in a manner that predisposes individuals to disease in adulthood. Chronic illness is thought to emerge as a function of the altered responses to environmental demand (stressors) in conjunction with an increased level of prevailing adversity. These models[11–14] are supported by research showing that low-LG prolonged activation of neural and hormonal responses to stressors can promote illness, and early environmental events influence the development of stress responses. In humans, physical or sexual abuse (or both), poor parental bonding and family dysfunction in early life increase endocrine and auto-

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nomic responses to stress in adulthood[14–19] as well as cognitive processing of potentially threatening stimuli.[20] There is evidence for comparable developmental effects in primates[10,21,22] and rodents,[23–27] albeit with models that rely on prolonged periods of separation of parent and offspring. Moreover, sustained exposure to elevated levels of stress hormones, including corticotrophin-releasing factor (CRF); catecholamines, most notably norepinepherine; and glucocorticoids can actively promote the development of a diverse range of high-risk conditions, such as visceral obesity, hypertension and insulin intolerance, or overt pathology, including diabetes, depression, anxiety disorders, drug addiction and multiple forms of coronary heart disease.[28–32] It is not difficult to understand the appeal of stress diathesis models. Because stress hormones regulate such a wide range of physiological processes, a very real strength lies in their ability to explain individual differences in vulnerability for specific diseases and the high level of comorbidity between brain-based disorders and cardiovascular/metabolic illness.

The relation between the quality of the early environment and health in adulthood appears to be mediated by parental influences on the development of neural systems that underlie the expression of behavioural and endocrine responses to stress.[33] There is strong evidence for such parental mediation in developmental psychology. For example, the effects of poverty on emotional and cognitive development are mediated by variations in parent–offspring interactions: if parental care factors are statistically controlled, there no longer remains any discernible effect of poverty on child development.[34,35] Such findings are not surprising. Poverty imposes considerable stress on the family unit, and stressors seriously compromise the quality of parental care.[1,36] In humans, high levels of maternal stress during the transition to parenthood are associated with depressed or anxious mood states and less sensitive parent–child interactions that, in turn, influence the quality of parent–child attachment.[37–39] Unstable or stressful environments, such as those prevailing under conditions of poverty, are associated with greater variability in the quality of infant–mother attachments.[40] Parents who experience poverty or other environmental stressors more frequently experience negative emotions such as irritability, depressed or anxious moods or both, which can lead to more punitive forms of parenting.[34,41,42] Reduced parental education, low income, multiple children, the absence of social support and single parenthood predict forms of parenting (verbal threats, pushing or grabbing, emotional neglect, overt physical abuse, and more controlling attitudes toward the child) that compromise cognitive development and result in more anxious and behaviourally inhibited children. In this review, we consider environmental effects occurring during the early postnatal period. There is, of course, considerable evidence for the effects of adversity on the mother and offspring during the prenatal period[43–47]; thus, the influence of adversity is best seen as being continuous, with effects through development at multiple genomic targets and influences on a wide range of functional outcomes. Prenatal adversity is also associated with increased hypothalamic-pituitary-adrenal (HPA) and autonomic responses to stressors.[43–51]

Support for the basic elements of stress diathesis models is compelling. Adversity during perinatal life alters development in a manner that seems likely to promote vulnerability, especially for stress-related diseases. Diathesis describes the interaction between development, including the potential influence of genomic variations, and the prevailing level of stress in predicting health outcomes. Such models could identify both the origins and the nature of vulnerability.

Maternal care in the rat: behavioural and HPA responses to stress

CRF systems furnish the critical signal for the activation of behavioural, emotional, autonomic and endocrine responses to stressors. There are 2 major CRF pathways regulating the expression of these stress responses. One is a CRF pathway from the parvocellular regions of the periventricular nucleus of the hypothalamus (PVNh) to the hypophysial–portal system of the anterior pituitary, which serves as the principal mechanism for the transduction of a neural signal into a pituitary–adrenal response.[52–56] In response to stressors, CRF, as well as cosecretagogues such as arginine vasopressin, are released from the PVNh neurons into the portal blood supply of the anterior pituitary, where the pituitary stimulates the synthesis and release of adrenocorticotropin hormone (ACTH). Pituitary ACTH, in turn, causes the release of glucocorticoids from the adrenal gland. CRF synthesis and release is subsequently inhibited through a glucocorticoid negative-feedback system mediated by both mineralocorticoid and glucocorticoid receptors in a number of brain regions including, and perhaps especially, the hippocampus.[57,58] For example, selective disruption of the glucocorticoid receptor gene in the hippocampus and cortex that is unique to adulthood results in negative feedback impairments and increased HPA activity.[59]

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 (icv) infusion of CRF increases extracellular noradrenaline levels.[60–63] The amygdaloid bed nucleus of the stria terminalis (BNST) CRF projection to the locus coeruleus[63–67] is also critical for the expression of behavioural responses to stress.[68–75] Hence, the CRF neurons in the PVNh and the central nucleus of the amygdala are important mediators of both behavioural and endocrine responses to stress.

We examine the relation between maternal care and the development of behavioural and endocrine responses to stress in the Long-Evans rat, using a model of naturally occurring variations in maternal behaviour over the first 8 days after birth.[76] We characterize individual differences in maternal behaviour through direct observation of mother–pup interactions in normally reared animals. These observations reveal considerable variation in pup licking (L) and grooming (G)[77] that includes both anogenital and nonanogenital licking. For the studies described here, high- and low-LG mothers are females whose scores on LG measures were

above (high) or below (low) 1 standard deviation above the mean for their cohort. High- and low-LG mothers do not differ in the amount of contact time with pups; therefore, differences in the frequency of LG do not occur simply as a function of time in contact with pups. High- and low-LG mothers raise a comparable number of pups to weaning, and there are no differences in the weaning weights of the pups, suggesting 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. Indeed, the frequency of pup LG is normally distributed across large populations of lactating female rats.[76]

These variations in maternal care are associated with stable individual differences in behavioural and neuroendocrine responses to stress in the offspring.[78–81] As adults, the offspring of high-LG mothers show reduced plasma ACTH and corticosterone responses to acute stress, compared with those of low-LG mothers. As mentioned above, circulating glucocorticoids act at glucocorticoid and mineralocorticoid receptor sites in corticolimbic structures, such as the hippocampus, to regulate HPA activity. Indeed, in several rodent models, downregulation of hippocampal glucocorticoid receptor (GR) levels is associated with increased HPA activity.[58] Such glucocorticoid feedback effects commonly target CRF synthesis and release at the level of the PVNh.[53] The high-LG offspring showed significantly increased hippocampal glucocorticoid receptor messenger ribonucleic acid (mRNA) expression, enhanced glucocorticoid negative feedback sensitivity and decreased hypothalamic CRH mRNA levels. Moreover, Liu and colleagues[79] found that the magnitude of the corticosterone response to acute stress was significantly correlated with the frequency of both maternal licking and grooming ($r = –0.61$) during the first week of life, as was the level of hippocampal glucocorticoid receptor mRNA and hypothalamic CRH mRNA expression (all $r > 0.70$). Although such studies more commonly focus on adult male offspring, the maternal effect on HPA responses to stress is also apparent in the adult female offspring.

An earlier study[82] reveals that the selective downregulation of GR levels in the hippocampus is sufficient to eliminate differences in HPA responses to acute stress derived from variations in early experience. Indeed, the results of recent studies suggest a direct relation between the effects of maternal care on hippocampal GR expression and those on HPA responses to acute stress. Direct infusion of RU38486, a GR antagonist, into the hippocampus eliminates the differences in corticosterone responses to acute stress in the offspring of high- and low-LG mothers.

Apparently, the maternal effect on HPA function is not limited to stress-induced activity.[83] The HPA axis shows a well-defined circadian rhythm, with the peak in activity occurring in the hours before the most active phase of the light–dark cycle. The adult offspring of low-, compared with high-, LG mothers show increased levels of both ACTH and corticosterone at the time of the normal circadian peak in activity (the latter portion of the light phase and early portion of the dark phase). HPA activity over the diurnal period is regulated by both mineralocorticoid and glucocorticoid receptors, depending on the phase of the cycle.[57] The adult offspring of high- and low-LG mothers differ in hippocampal expression of glucocorticoid but not mineralocorticoid receptor expression. Interestingly, influence of the GR activity is most apparent during the diurnal peak in basal HPA activity, which corresponds to the time when differences in basal HPA activity as a function of maternal care are apparent. Mineralcorticoid receptor influence prevails during the nadir in HPA activity, a time when there are no differences in basal HPA activity.

Prolonged periods of maternal separation in the neonatal rat result in sustained alterations in the CRF system, including increased CRF expression in the PVNh and the amygdala, and enhanced behavioural responses to stress.[24,84] The CRF system of the BNST/amygdala–locus coeruleus is also altered as a function of normal variations in maternal care in the rat. The offspring of the high-LG mothers showed decreased CRF receptor levels in the locus coeruleus and increased gamma amino butyric acid ($GABA_{A)}$/benzodiazepine (BZ) receptor levels in the basolateral and central nuclei of the amygdala, and in the locus coeruleus[78,85] and decreased CRF mRNA expression in the central nucleus of the amygdala. BZ agonists suppress CRF expression in the amygdala,[86] and the GABA system is an important modulator of activity within the amygdala in tests of fear conditioning.[87] Predictably, stress-induced increases in PVNh levels of noradrenaline that are normally stimulated by CRF were significantly higher in the low-LG offspring.[88] The offspring of the high- and low-LG mothers also differ in behavioural responses to novelty.[85,89] As adults, the offspring of the high-LG mothers showed decreased startle responses, increased open-field exploration and shorter latencies to eat food provided in a novel environment. The offspring of low-LG mothers also show greater burying in the defensive burying paradigm,[90] which involves an active response to a threat.

Maternal care during the first week of life is associated with stable individual differences in $GABA_A$ receptor subunit expression in brain regions that regulate stress reactivity. The adult offspring of high-LG mothers show significantly higher levels of $GABA_A$/BZ receptor binding in the basolateral and central nuclei of the amygdala and the locus coeruleus. These findings provide a mechanism for increased GABAergic inhibition of amygdala–locus coeruleus activity. More recent studies[85] illustrate 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. The offspring of high-LG mothers show increased levels of mRNAs for the $\gamma 1$ and $\gamma 2$ subunits that contribute to the formation of a functional BZ binding site. Such differences are not unique to the γ subunits. Levels of mRNA for the $\alpha 1$ subunit of the $GABA_A$/BZ receptor complex are significantly higher in the amygdala and locus coeruleus of offspring of high, compared with low, LG mothers. The $\alpha 1$ subunit appears to confer higher affinity for GABA, providing the most efficient form of the $GABA_A$ receptor complex, through increased receptor affinity for GABA. A direct effect of maternal care is revealed in the results of cross-fostering

studies[85] showing that the expression of the γ2 or α1 subunits in the amygdala or the locus coeruleus of animals born to low-LG mothers but reared by high-LG dams is comparable with that of the normal offspring of high-LG mothers; the converse is also true.

Adult offspring of the low-LG mothers actually show increased expression of the mRNAs for the α3 and α4 subunits in the amygdala and locus coeruleus. Interestingly, $GABA_A$/BZ receptors composed of the α3 and α4 subunits show a reduced affinity for GABA, compared with those bearing the α1 subunit. Moreover, the α4 subunit does not contribute to the formation of a BZ receptor site. These differences in subunit expression are tissue specific; no such differences are apparent in the hippocampus, hypothalamus or cortex. Thus, differences in $GABA_A$/BZ receptor binding are not simply caused by a deficit in subunit expression in the offspring of the low-LG mothers, but are apparently an active attempt to maintain a specific $GABA_A$/BZ receptor profile in selected brain regions.

The critical question concerns the relation between these profiles and fear-related behaviour. Studies with animals bearing mutations of various $GABA_A$/BZ receptor subunits suggest that mutations of the γ2 subunit do indeed lead to decreased BZ receptor binding and increased fearfulness. Mice that are heterozygous for the γ2 null mutation (γ 2+/− mice) are viable and fertile (the homozygous null mutation is lethal) and display enhanced behavioural inhibition toward aversive stimuli and heightened responsiveness in fear conditioning.[90,91] However, among the alpha subunits, it is α2 and not α1 that has been more directly linked to the anxiolytic effects of BZ treatment; the α1 subunit is linked to the hypnotic effects (see Rudolph and Mohler[92] for a review). Thus, the precise cause–effect relation between the effects of maternal care on $GABA_A$/BZ receptor subunits and fear remains to be clearly defined. Here, and throughout this area of research, an understanding of the importance of the effects of early environment on gene expression requires a more precise definition at the level of function. However, a recent study[92] revealing differences in BZ sensitivity between the adult offspring of high- and low-LG mothers suggests that maternal effects on the expression of $GABA_A$/BZ receptor subunits are of functional importance. In this study, there was a significantly greater anxiolytic effect of BZ treatment in the adult offspring of high, compared with low, LG mothers.

Individual differences in behavioural and neuroendocrine responses to stress in the rat are associated with 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 mothers are more fearful and show increased HPA responses to stress compared with high-LG dams.[89] Individual differences in stress reactivity are apparently transmitted across generations: fearful mothers beget more stress-reactive offspring. 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 behaviour may simply be an epiphenomenon and not causally related to the development of individual differences in stress responses. The issue is not one of inheritance, but mode of inheritance.

The results of cross-fostering studies provide evidence for a nongenomic transmission of individual differences in stress reactivity.[89] The critical groups of interest are the biological offspring of low-LG mothers fostered onto high-LG dams and vice versa. The results are consistent with the idea that variations in maternal care are causally related to individual differences in the behaviour of the offspring. The biological offspring of low-LG dams reared by high-LG mothers are significantly less fearful under conditions of novelty than are the offspring reared by low-LG mothers, including the biological offspring of high-LG mothers.[89] Subsequent studies reveal similar findings for hippocampal glucocorticoid receptor expression and for the differences in both the α1 and γ2 $GABA_A$ receptor subunit expression in the amygdala.[85] These findings suggest that individual differences in patterns of gene expression and stress responses can be directly linked to maternal care in the first week of life.

Maternal care and hippocampal development

Maternal care influences the activity of endocrine systems that determine metabolic states and growth in neonates. Tactile stimulation from the mother stimulates the release of growth hormone. Kuhn and colleagues[93] showed that prolonged separation from the dam resulted in a dramatic decrease in plasma growth hormone levels. Such effects were reversed with experimental stroking with a brush, mimicking the tactile stimulate derived from maternal LG. Suchecki and colleagues[94] found a comparable effect on pup HPA activity. Separation increases glucocorticoid levels in the pup, and these effects attenuated with stroking and were completely eliminated when stroking was combined with feeding. Under conditions of regular maternal contact, the nutritional and thermoregulatory demands of the pup are met by the mother. In her absence, the pup increases HPA activity, activating a catabolic state that serves to mobilize energy reserves. Resources that would otherwise be directed toward growth, under the direction of growth hormone, are now required for survival. A comparable situation occurs in the brain. Maternal deprivation decreases the expression of brain-derived neurotrophic factor (BDNF) expression in neonates.[25] The results of these studies suggest that tactile stimulation derived from maternal LG can promote an endocrine or paracrine state that fosters growth and development. Indeed, Huot and colleagues[26] reported decreased mossy fibre density in the hippocampus of adult animals as a function of repeated and prolonged maternal separation in infancy. Similarly, Mirescu and others[95] reported that maternal separation in early life alters hippocampal neurogenesis. A decrease in neuron proliferation characteristic of the adult hippocampus is significantly reduced in adult animals as a function of maternal separation over the first weeks of life.

Studies of maternal separation reveal a potential influence of variations in mother–offspring interactions on neural development. To examine the effects of natural variations in maternal LG, we examined hippocampal gene expression us-

ing complementary DNA (cDNA) rays.[96] The analyses of the array data revealed major classes of maternal effects on hippocampal gene expression in postnatal day 6 offspring, including 1) genes related to cellular metabolic activity (glucose transporter, cFOS, cytochrome oxidase and low-density lipoprotein receptor; 2) genes related to glutamate receptor function, including effects on the glycine receptor and those mentioned for the *N*-methyl-D-aspartic acid (NMDA) receptor sub-units; and 3) genes encoding for growth factors, including BDNF, basic fibroblast growth factor (bFGF) and β-NGF. In each case, expression was > 2-fold higher in hippocampal samples from offspring of high, compared with low, LG mothers. A subsequent cDNA array analysis comparing expression profiles in the hippocampus from the adult offspring of high- and low-LG mothers[97] found that constuitive expression of genes associated with synaptic plasticity, such as the subunits of the various glutamate receptors and those involved in the mitogen-activated protein kinase (MAPK) pathways, were elevated in the offspring of high-LG mothers (i.e., > 1.5-fold increases).

Essentially, maternal care increases genes that provide metabolic support, mediates experience-dependent neuronal activation and supports the growth and survival of synapses. Not surprisingly, variations in maternal care are associated with individual differences in the synaptic development of the hippocampus, including neural systems that mediate learning and memory. As adults, the offspring of high-LG mothers show enhanced spatial learning or memory or both in the Morris water maze and in object recognition.[98–100] Performance in both tasks is dependent on hippocampal function,[101–103] and maternal care alters the synaptic development of the hippocampus. At either day 18 or day 90, there are significantly increased levels neural cell adhesion molecule (N-CAM) or synaptophysin-like immunoreactivity on Western blots in hippocampal samples from the offspring of high, compared with low, LG mothers, suggesting increased synapse formation or survival. Subsequent studies provided evidence for a maternal effect on neuron survival in the hippocampus.[104] Bromodeoxyuridine (BrdU) injections made on day 7 of life revealed group differences in neuronal proliferation. However, at 21 and 90 days of life, there were significantly more BrdU-labelled cells in the offspring of high-, compared with low-, LG mothers.

Recently, Champagne and colleagues[105] reported on the results of a golgi-staining study of the morphology of hippocampal neurons in adult animals. Although the branch length of individual dendrites was comparable, the number of dendritic branches was significantly greater in the adult offspring of high-LG mothers. The enhanced dendritic arborization suggests an increased capacity for synaptic plasticity in the adult offspring of high-LG mothers. Long-term potentiation is an electrophysiological model of synaptic plasticity and was assessed for 60 minutes in response to tetanic stimulation in the Schaffer collateral pathway. The results show a significantly lower percentage of long-term potentiation (LTP) in low-LG offspring relative to high-LG offspring. These findings are in line with previous findings of defective synaptic plasticity in low-LG offspring[99] and extend this phenomenon to another subfield of the hippocampus.

The influence of the hippocampus in spatial learning is thought to involve, at least in part, cholinergic innervation emerging from the medial septum.[106] In the adult offspring of the high-LG mothers, there was increased hippocampal choline acetyltransferase (ChAT) activity and acetylcholinesterase staining, as well as increased hippocampal basal and K^+–stimulated acetylcholine release.[98] These findings suggest increased cholinergic synaptic number in the hippocampus of the offspring of high-LG mothers. Hippocampal BDNF mRNA levels are elevated in the high-LG offspring on day 8 of life.[98] BDNF is commonly associated with the survival of cholinergic synapses in the rat forebrain[107–109]; for example, there is decreased hippocampal ChAT activity in BDNF knockdown mice.

The expression of BDNF is regulated by NMDA receptor activation, and tactile stimulation increases NMDA receptor expression in the barrel cells of mice.[110] 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 mothers at day 8 of life,[98] and these effects on gene expression are associated with increased hippocampal NMDA receptor binding.

Naturally occurring variations in maternal LG and arched-back nursing are associated with the development of cholinergic innervation to the hippocampus, as well as differences in the expression of NMDA receptor subunit mRNAs. There is also increased hippocampal NR1 mRNA expression in the adult offspring of high-LG mothers. These findings provide a mechanism for the differences observed in spatial learning and memory in adult animals. In the adult rat, spatial learning and memory is dependent on hippocampal integrity; lesions of the hippocampus result in profound spatial learning impairments. Moreover, spatial learning is regulated by both cholinergic or NMDA receptor activation or NR1 subunit knockout.[111–113] These findings suggest that maternal care increases hippocampal NMDA receptor levels, resulting in elevated BDNF expression and increased hippocampal synaptogenesis and, thus, enhanced spatial learning in adulthood. These results are also consistent with the idea that maternal behaviour actively stimulates hippocampal synaptogenesis in offspring through systems known to mediate experience-dependent neural development.

An NR2B-specific receptor antagonist, ifenprodil, infused directly into the CA1 region of the hippocampus completely eliminates the group differences in the Morris water maze.[100] The NMDA receptor complex, and the NR2B subunit in particular, is interesting because of its importance in synaptic plasticity and hippocampal-dependent learning and memory,[102] and ifenprodil blocks the effects of postweaning environmental enrichment on spatial learning and memory. Transgenic mice overexpressing the NR2B subunit exhibit enhanced hippocampal LTP and improved learning and memory compared with wild-type controls. After exposure to environmental enrichment, wild-type mice show an overall improvement in contextual and cued conditioning, fear extinction and novel object recognition learning, with little or no effect of enrichment on the performance of the NR2B transgenic mice.[103] One explanation for these findings is that,

in animals where there is an overexpression of the NR2B subunit, environment provides no further "gain of function." The adult offspring of low-LG mothers reared under conditions of environmental enrichment show increased hippocampal NR2B expression and synaptic density as well as performance in the Morris water maze test or object-recognition test that is comparable to that of the adult offspring of high-LG mothers. Predictably, the adult offspring of high-LG mothers, which normally exhibit increased NR2B expression, are unaffected by environmental enrichment.

These findings suggest that maternal care in the rat directly influences hippocampal development by effecting the expression of genes involved in both neuron survival and synaptic development. The group differences in performance in the Morris water maze are consistent with a maternal effect on cognitive performance in adulthood. However, the Morris water maze is a model of escape learning that, by definition, involves an aversive component, which provides the motivation for escape. The water maze is an interesting task for this discussion because it provides an opportunity to examine cognitive performance under stressful conditions. In sequence, the animal must contend with removal from the home cage; transport to the testing area; placement into the pool of water, murky at that; and the uncertainty at each stage of testing. Initially, most animals behave in a manner similar to that of an open-field test, circling the perimeter and remaining close to the walls (i.e., thigmotaxis). There is little opportunity for learning so long as the animal refuses to enter the centre area of the swim maze where the platform is located. The tendency to remain close to the walls and reluctance to enter the centre area is commonly associated with a fear response to the environment. Not surprisingly, thigmotaxis is significantly more prevalent in the offspring of low, compared with high, LG mothers. The difference in thigmotaxis is reversed with postweaning environmental enrichment.[104] Moreover, Smythe and colleagues[114,115] show that blockade of hippocampal cholinergic input results in increased fear behaviour under conditions of novelty. The effect is blocked with acute benzodiazepine administration. The offspring of low-LG mothers show decreased hippocampal cholinergic innervation, which might explain the increased thigmotaxis and thus the impaired performance in the Morris water maze. These findings underscore the complex relations between emotional and cognitive systems in determining the behaviour of animals in stressful conditions.

Maternal effects on gene expression

These studies reflect the potential consequences for neurodevelopment of maternal regulation of gene expression in the pup. An obvious question here concerns how early experience might serve to program gene expression. Variations in maternal care over the first week of life alter hippocampal glucocorticoid receptor expression and HPA responses to stress. These effects endure well beyond weaning and suggest a stable influence of maternal care on the development of individual differences in stress responses. The critical question concerns the mechanisms whereby these maternal effects, or other forms of environmental programming, are sustained over the lifespan of the animal. The effect of maternal care on glucocorticoid receptor expression in the hippocampus appears to involve an epigenetic modification of the DNA and, specifically, at the exon 1_7 promoter of the glucocorticoid receptor. The neural-specific exon 1_7 promoter is significantly more active in the hippocampus of adult offspring of high, compared with low, LG mothers.[116,117] The exon 1_7 promoter contains a consensus-binding sequence for nerve growth factor-induced protein-A (NGFI-A) and NGFI-A can activate transcription through the 1_7 promoter.

The NGFI-A consensus sequence contains 2 CpG dinucleotides, and maternal care is associated with an alteration in the methylation of the 5′ CpG site of the NGFI-A site.[80] The 5′ cytosine is heavily methylated in the offspring of low-LG mothers and rarely in those of high-LG dams. This pattern is reversed with cross-fostering, suggesting a direct effect of maternal care. Methylation of the 5′ CpG significantly decreases NGFI-A binding to the exon 1_7 promoter in gel shift assays, and the results of chromatin immunoprecipitation assays reveal greater in vivo NGFI-A binding to the exon 1_7 promoter sequence in the hippocampus of high, compared with low, LG mothers.[80,117] The findings suggest that maternal care alters the methylation status of the NGFI-A consensus sequence and, thus, the NGFI-A binding to the exon 1_7 promoter.

DNA methylation inhibits transcription factor binding through alterations in chromatin structure, which gates the accessibility of promoters to transcription factors.[118,119] Histone acetylation at lysine (K)-9 residue of H3 and H4 histones is a well-established marker of active chromatin, transcription factor binding and gene expression. Acetylation of the histone tails neutralizes the positively charged histones, which disrupts histone binding to negatively charged DNA, opens up the histone–DNA complex and thus promotes transcription factor binding. Weaver and others[80,117] found that maternal effect on DNA methylation is associated with increased histone acetylation at the lysine 9 residue of histone 3 (H3) associated with the exon 1_7 glucocorticoid receptor promoter and increased interaction of NGFI-A with the promoter sequence. Cytosine methylation can stably silence gene expression through the binding of methylated DNA binding proteins, which bind with a complex that includes histone deacetylase (HDAC). HDAC, in turn, prevents histone acetylation, stabilizing the tight histone–DNA relation and preventing transcription factor binding. Thus, in the adult offspring of low-LG mothers, central infusion of trichostatin-A, a potent HDAC inhibitor, permits greater H3 acetylation and increased NGFI-A binding to the exon 1_7 promoter. Predictably, the increased NGFI-A binding to the exon 1_7 promoter results in greater glucocorticoid receptor expression and, most importantly, eliminates the group difference in HPA responses to stress.

These findings suggest that sustained effects of maternal care on gene expression are caused by alterations of DNA methylation and chromatin structure at relevant promoter sites. A simple developmental time course study[80] reveals that the difference in cytosine methylation of the NGFI-A

consensus sequence actually involves a process of demethylation.[118,119] On the day after birth, methylation of the NGFI-A consensus sequence is comparable in the offspring of high- and low-LG mothers. Between postnatal days 1 and 6, the difference at the 5′, but not the 3′ cytosine becomes apparent, suggesting an active demethylation process.[118,120] It is over the same period that the high- and low-LG mothers differ in maternal behaviour.[77,78]

Conclusions

The dissolution of epigenetic marks, such as cytosine methylation, through processes such as demethylation reflects a costly energy investment. Demethylation requires specific enzymatic machinery and breaking carbon-carbon bonds. The end point appears to be that of sustained changes in gene expression that reflect variations in maternal care. So the obvious question is — why bother? We think that maternal effects represent a developmental strategy whereby the defensive responses of the offspring are refined in response to the prevailing level of environmental demand. In mammals, the relevant signal that predicts the level of environmental demand is the behaviour of the parent. Indeed, we use the term "developmental strategy" here in a descriptive sense, since the strategy may be seen as emerging from a strategy on the part of the offspring (i.e., use the signals of the parent to forecast environmental demand[121]) or the parent (i.e., signal the offspring in a manner that influences the development of defensive responses). These need not be considered as mutually exclusive options. The crucial assumption is that the result confers some advantage onto the offspring with respect to survival and reproduction.

We propose that adversity in mammals alters parent–offspring interactions in a manner that is designed to increased endocrine, cognitive and emotional responses to stress. In the rat, gestational stress is associated with decreased maternal LG[122,123] and increased stress reactivity in the offspring.[122] In the macaque, stress imposed on lactating females decreases the quality of mother–infant interactions[124] and increases endocrine and behavioural responses to stress.[125,126] In the rat, decreased maternal LG is associated with increased fearfulness, enhanced HPA responses to stress and impaired performance on attentional tasks and tests of declarative learning or memory under stressful conditions. These effects appear to be mediated by maternal effects on gene expression in relevant brain regions. We suggest that such effects produce an increased state of preparedness of defensive systems. This could be of particular importance in the time after weaning and independence from the parent when mortality rates are extremely high in most mammals. Considering the adaptive value of behavioural and endocrine responses to stress, such a bias may be especially important for an individual functioning under conditions of increased adversity. If this is the case, then we are better to consider functional differences in developmental outcomes under conditions of adversity as reflecting alternative phenotypes rather than impairments in development.

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JPN's Top Ten Articles, June 2007
(based on Web views on PubMed Central)

1. **Efficacy of escitalopram in the treatment of major depressive disorder compared with conventional selective serotonin reuptake inhibitors and venlafaxine XR: a meta-analysis**
   Kennedy et al
   J Psychiatry Neurosci 2006;31(2):122-31
2. **Platelet serotonin levels support depression scores for women with postpartum depression**
   Maurer-Spurej et al
   J Psychiatry Neurosci 2007;32(1):23-9
3. **Treatment of primary insomnia with melatonin: a double-blind, placebo-controlled, crossover study**
   Almeida Montes et al
   J Psychiatry Neurosci 2003;28(3):191-6
4. **"Missing" links in borderline personality disorder: loss of neural synchrony relates to lack of emotion regulation and impulse control**
   Williams et al
   J Psychiatry Neurosci 2006;31(3):181-8
5. **Citalopram - a review of pharmacological and clinical effects.**
   Bezchlibnyk–Butler et al
   J Psychiatry Neurosci 2000;25(3):241-54
6. **Eating disorder and obsessive–compulsive disorder: neurochemical and phenomenological commonalities**
   Jarry and Vaccarino
   J Psychiatry Neurosci 1996;21(1):36-48
7. **Electroconvulsive shock enhances striatal dopamine D_1 and D_3 receptor binding and improves motor performance in 6-OHDA-lesioned rats**
   Strome et al
   J Psychiatry Neurosci 2007;32(3):193-202
8. **Depression in acute stroke**
   Caeiro et al
   J Psychiatry Neurosci 2006;31(6):377-83
9. **Reduced hippocampal volume correlates with executive dysfunctioning in major depression**
   Frodl et al
   J Psychiatry Neurosci 2006;31(5):316-25
10. **Vulnerability for apoptosis in the limbic system after myocardial infarction in rats: a possible model for human postinfarct major depression**
    Wann et al
    J Psychiatry Neurosci 2007;32(1):11-6