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Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights

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Parental care plays an important role in the emotional and cognitive development of the offspring. Children who have been exposed to abuse or neglect are more likely to develop numerous psychopathologies, while good parent–infant bonding is associated with improved resiliency to stress. Similar observations have also been reported in non-human primates and rodents, suggesting that at least some neurodevelopmental aspects of parent–offspring interactions are conserved among mammals and could therefore be studied in animals. We present data to suggest that frequency of licking and grooming provided by the dam during a critical period in development plays an important role in modifying neurodevelopment. These findings are examined in the broader context in which exposure to other sensory modalities such as vision or hearing during a specific period in development shapes brain development with functional consequences that persist into adulthood. We also discuss recent rodent work showing that increased frequency of licking and grooming provided by the dam during the first week of life is associated with changes in DNA methylation of promoter elements that control expression of these genes and behavior. The stability of DNA methylation in postmitotic cells provides a possible molecular scaffold by which changes in gene expression and behavioral traits induced by postnatal maternal care are maintained throughout life. Finally, the relevance of findings reported in rodents to those noted in non-human primates and humans are assessed and the research and clinical implications of these observations for future work are explored. **Keywords:** Family factors, hormones, parent–child interaction, maternal care, epigenetic, DNA methylation.

Childhood abuse or neglect is associated with increased vulnerability for several psychopathologies such as anxiety, mood disorders, poor impulse control, and psychosis that persist throughout life (Bebbington et al., 2004; Freyd et al., 2005; Heim & Nemeroff, 2002; Mullen, Martin, Anderson, Romans, & Herbison, 1996). Importantly, sequelae of early-life stress are not restricted to psychiatric vulnerabilities, but are also associated with increased risk for several other medical morbidities such as obesity and cardiovascular diseases (Felitti et al., 1998). Several observations suggest that at least some of the long-tem effects of early-life stress are mediated by poor levels of parent–infant bonding and decreased parental investment during early life. For example, poor parental bonding not considered abuse or neglect is also associated with increased risk for several psychological vulnerabilities (Canetti, Bachar, Galili-Weisstub, De-Nour, & Shalev, 1997), and randomized control interventions aimed at improving parental care demonstrate improved behavioral outcomes and cognitive performance that persist for years (D. Olds et al., 1998; D.L. Olds, Kitzman et al., 2004; D.L. Olds, Robinson et al., 2004). Parental care also affects similar aspects of behavior and cognition in non-human primates and in rodents (Barr et al., 2003; Meaney, 2001; Suomi, 2003). Indeed, so-called 'maternal effects' are apparent on defensive responses to threat and reproductive strategies in a remarkable array of species, ranging literally from plants to mammals (Cameron et al., 2005; Mousseau & Fox, 1998). This apparently conserved influence of parental care on neurodevelopment suggests that such animal models could be used to further understand the molecular mechanisms that guide these processes. The two aims of this review are: first, to present recent progress in our molecular understanding of how parental care influences brain development in a manner that persists throughout life, and second, to discuss clinical and research implications of these findings.

We divided this review into three main sections. In the first section, we summarize evidence obtained mainly in rodents to demonstrate that tactile stimulation provided by the dam during the first week of life appears to play an important role in establishing diverse and stable behavioral phenotypes in the offspring. These findings are also discussed in the broader context of experimental paradigms such as the monocular deprivation and the development of the auditory cortex in which sensory input during early life shapes neurodevelopmental trajectory in a manner that persists throughout life. In the second section, we present recent studies suggesting that a

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pattern of DNA methylation established by maternal care early in life provides the molecular stability necessary to maintain transcriptional regulation of genes that control behavior throughout life. We discuss the controversy around the possibility of postmitotic DNA methylation and demethylation, and propose that DNA methylation and other stable chromatin rearrangements represent a new field in molecular neuroscience with promising potential to expand our understanding of how stable behavioral phenotypes are established and maintained. We also briefly examine the role of extracellular matrix deposition in maintaining the visual sequelae of early-life monocular deprivation to demonstrate another mechanism by which changes in brain functioning early in life are maintained throughout life. Finally, the ability of maternal behavior to affect several behavioral phenotypes in the offspring, including maternal care, provides a mechanism by which acquired and stable behavioral traits can be propagated across generations via epigenetic modifications of DNA. The research and clinical implications associated with this form of transmission are examined in the third section of this review.

Historical background

Studies in the 1950s by Denenberg, Levine and others showed that brief (3–15 min) daily removal of the pups from the dam during the first three weeks of life has profound and long-lasting effects on stress reactivity throughout life (Meaney, 2001). More specifically, adult animals handled during the postnatal period showed increased exploratory behavior in novel environments and blunted hypothalamic-pituitary axis (HPA) responses to stress as compared to nonhandled animals (Hess, Denenberg, Zarrow, & Pfeifer, 1969; Levine, Alpert, & Lewis, 1957; Levine, Haltmeyer, Karas, & Denenberg, 1967; Meaney, Aitken, Sharma, & Viau, 1992; Meaney, Aitken, Viau, Sharma, & Sarrieau, 1989). Further work showed that handling during the first week of life, but not during postnatal days 14–21, is as effective as handling for the entire first three weeks of life (Hess et al., 1969; Meaney & Aitken, 1985). These data indicate that brief separation during a specific period in development (i.e., the first week of life) is somehow necessary to alter the animals' response to stress in adulthood.

Part I: Tactile stimulation provided by the dam during the first week of life is associated with long-term changes in behavior

The enduring effects of postnatal handling on stress reactivity raise several questions: Why is handling effective in the first week of life, but less so thereafter? What environmental aspect of the brief separation is responsible for the behavioral sequelae of handling? What molecular events are necessary to induce neurodevelopmental changes associated with handling? How do such 'downstream' molecular changes influence behavior and stress reactivity in adulthood? Here we address the first question and present data that suggest that tactile stimulation provided by the dam plays an important role in establishing the long-term sequelae of handling.

Like all other mammals, the rodent pup is dependent on the mother for survival. It is therefore not surprising that the pup is capable of detecting separation from the dam and mounting an adaptive response to the absence of the mother. Such a response includes ultrasonic vocalization, attempting to relocate the nest, the release of stress hormones such as corticosterone, and inhibition of several anabolic pathways associated with development and growth (Hofer, 1994; Kuhn & Schanberg, 1998; Levine, 1975). The increase in ultrasonic vocalization, for example, helps the dam to locate the separated pup while corticosterone release is thought to be important for gluconeogenesis necessary to maintain adequate glucose levels in the absence of exogenous food supply. Work from Hofer's, Schanberg's, and Levine's groups characterized the aspects of maternal care that appear to mediate this complex response to separation. For example, loss of thermal support provided by the dam appears to be important in inducing ultrasonic vocalization, while loss of active tactile stimulation such as licking and grooming appears to initially inhibit secretion of growth hormone and DNA synthesis, followed by activation of ACTH release after a more prolonged period of separation (Kuhn & Schanberg, 1998; Levine, 2001). This initial work focused mainly on changes in pup physiology associated with maternal separation with little attention to the effect of pup removal on alteration in maternal physiology and behavior. Formal observations showed that when the dam is reunited with the pups, it immediately approaches the pups to restore maternal care followed by a sustained increase in several forms of maternal behaviors. These behaviors include increased licking and grooming (LG) and crouching over the pups in an active form of nursing known as arched-back nursing (ABN) (Lee & Williams, 1974; Liu et al., 1997). It is currently unknown if the change in maternal behavior seen in handling is due to physiological changes that occur in the mother during the separation period, or to the ability of the reunited pups to elicit more maternal care, or both.

Since handling induces physiological changes in the pup and also alters maternal care, it was unclear whether the former, the latter, or both were responsible for the behavioral and physiological sequelae of handling. The answer to this question is still unknown, but a growing body of evidence presented below indicates that at least some of the long-term consequences of handling are likely to be mediated

by changes in tactile stimulation provided by the dam during the first week of life (Levine, 1975; Smotherman, 1983).

First, treatment of the mother with a benzodiazepine, which prevents the compensatory increase in pup LG following brief mother–pup separations, completely blocks the handling effect on exploratory behavior (D'Amato, Cabib, Ventura, & Orsini, 1998), providing support for the maternal mediation hypothesis.

Second, in a series of elegant experiments Denenberg, Zarrow, and Rosenberg showed that increased intensities of maternal care early in life reduce stress reactivity in mice. The initial observations demonstrated that mice raised by lactating rats are less fearful compared to those raised by lactating mice (Denenberg, Rosenberg, Paschke, Hess, & Zarrow, 1968). To determine whether this effect was due to differences in milk composition or maternal care between the two species, the authors compared fearful responses in mice offspring raised under three different conditions with lactating mouse dams present in all three groups. Postpartum thalactomized rats (i.e., nipples were surgically removed) were added to the first group, virgin rats to the second, and no additional rat maternal care in the control group. The authors reported that the thalactomized postpartum dams provided higher levels of maternal care compared to inexperienced virgin females, which in turn provided higher levels of maternal care compared to the control group. Since both the thalactomized and the virgin females were unable to nurse the pups, any differences in behavioral outcome in the offspring would be attributed to different levels of maternal care they received during the postnatal period. Indeed, mice that received the highest levels of maternal care (i.e., those raised in the presence of postpartum thalactomized rat) were the least fearful and secreted the lowest levels of corticosterone in response to stress, while offspring exposed to the lowest levels of maternal care (i.e., those raised in the absence of any additional help) were the most fearful and secreted the highest levels of corticosterone in response to stress. Moreover, there was a significant correlation between the level of maternal care provided during the postpartum period ($r = -.61$, $p < .01$) and fearful behavior in the offspring (Rosenberg, Denenberg, & Zarrow, 1970). This work demonstrates that increased levels of maternal care that are independent of maternal nursing are sufficient to program fearful responses in adulthood. It is important to note, however, that these experiments do not exclude the possibility that milk composition or some other aspect of nursing may also play an important role in pup development under normal conditions. In fact, Levine's work has demonstrated that milk provided by the mother plays an important role in inhibiting the ability of the adrenal gland to secrete corticosterone in response to ACTH stimulation (Levine, 2001).

Third, we characterized maternal behavior in Long-Evans hooded rats and reported large variability in levels of maternal care between individual dams (F.A. Champagne, Francis, Mar, & Meaney, 2003). Quantitative analysis of maternal behavior in this strain shows that variability in maternal care is restricted to frequency of LG and ABN with high correlations between these two forms of maternal behavior. Thus, dams that show a high frequency of LG also spend more time nursing their pups in the arched-back position (Caldji et al., 1998). Importantly, however, dams that show a high frequency of LG-ABN do not differ from low LG-ABN dams in other forms of maternal behavior such as overall time they spend in contact or nurse the pups. Moreover, differences in frequency of LG-ABN are normally distributed within the population with some dams showing almost three times more frequent LG-ABN as compared to others. Dams that are one standard deviation above the mean are labeled as high LG-ABN and those that are one standard deviation below the mean are considered low LG-ABN dams. Finally, frequency of LG-ABN subsides over the course of the first ten days after birth (Gubernick & Alberts, 1983) at which time no difference in LG-ABN is observed between high- and low-LG-ABN dams (F. A. Champagne et al., 2003).

We predicted that if frequency of LG-ABN is responsible for the behavioral outcome of handling, then offspring raised by high LG-ABN dams will be less fearful compared to those raised by low LG-ABN dams. Indeed, offspring raised by high LG-ABN dams show decreased HPA activation in response to stress (Liu et al., 1997) and were behaviorally less fearful in exploring a novel environment compared to those raised by low LG-ABN (Caldji et al., 1998). Importantly, these changes persist into the adult life of the animal. Moreover, almost all the changes in gene expression found in association with handling have also been seen in offspring exposed to high levels of LG-ABN early in life, suggesting that the two paradigms share many neurodevelopmental pathways (see Table 1 for details). Finally, the observation that differences in LG-ABN were restricted to the first week of life is remarkably similar to the time frame needed for handling to exert its long-term effects. Importantly, this developmental period also overlaps with the critical period in which tactile stimulation defines barrel receptor representation in the primary somatosensory cortex (Fox, 1995). Together, these data suggest that the developing rodent brain is exquisitely sensitive to tactile input during the first week of life and that different levels of tactile input provided during this period program neurodevelopment with long-lasting consequences on brain functioning.

Fourth, work from several groups has demonstrated that stroking the pups with a fine paintbrush affects HPA reactivity, cognition, and maternal care in a manner that resembles those of handling and

Table 1 Handling and postnatal maternal care both affect expression of many genes that control diverse array of behavioral phenotypes

Behavioral phenotype	Gene	Handling	LG-ABN
Stress reactivity	GR (hippocampus)	H > NH	High > low
	CRF (hypothalamus)	H < NH	High < low
	CRF receptor (amygdala)	H < NH	High < low
	GABA-A receptor $\gamma 2$ subunit (amygdala, LC, NTS)	H > NH	High > low
Cognition	NMDA receptor subunits NR1, NR2A, NR2B, (hippocampus)	Not known	High > low
	Synaptophysin (hippocampus)	Not known	High > low
	Acetylcholine esterase (hippocampus)	Not known	High > low
Maternal phenotype	ER- (MPOA)	Not known	High > low
	Oxytocin receptor (MPOA)	Not known	High > low

Abbreviations: CRF-coriticotropin-releasing factor, ER–Estrogen receptor alpha, GR-glucocorticoid receptor, H-handling, NH-non handled animals, MPOA-medial preoptic area. NMDA-N-methyl-D-aspartate, LC-locus ceruleus, NTS- nucleus tractus solitarius. Based on the following references: (Caldji et al., 2004; Caldji et al., 1998; F. Champagne et al., 2001; Liu et al., 2000; Liu et al., 1997).

exposure to high LG-ABN dams (see full discussion on the effects of LG on cognition and maternal behavior in the offspring in the sections below). Levine and his group showed that unlike adult animals, saline injection in pups is associated with a very small elevation in corticosterone blood levels. However, saline injection administered after a 24-hour period of maternal separation is associated with a significant increase in HPA reactivity in these pups. To investigate the role of maternal tactile stimulation in this alteration of HPA reactivity, the authors stroked the separated pups with a fine paintbrush during the separation period and showed that this manipulation was associated with blunted ACTH secretion in response to saline injection in these pups (Suchecki, Rosenfeld, & Levine, 1993). Jutapakdeegul et al. (2003) also report that stroking pups with a brush over the first week of life results in increased hippocampal glucocorticoid receptor (GR) expression in adulthood [Jutapakdeegul, Casalotti, Govitrapong, & Kotchabhakdi, 2003; see below for more details on the effect of postnatal maternal care on GR expression in the hippocampus]. Similarly, Saul Schanberg and his group showed that 8-day-old pups exposed to a single episode of 4-hour maternal separation show a dramatic decrease in growth hormone secretion and increased corticosterone levels. These alterations could be reversed by stroking the pups, but not by other tactile manipulations such as pinching, light stroking, vestibular stimulation or limb movements (Pauk, Kuhn, Field, & Schanberg, 1986). Finally, Alison Fleming's group recently developed an artificial rearing system in rats in which 3-day-old pups are removed from the dam, fed through a gastric cannula, and are reared at a control temperature in the complete absence of a dam. Using this procedure Fleming's group has shown that as adults, offspring raised by this artificial rearing have significant deficits in several cognitive tasks and are low LG dams as compared to control siblings raised with a mother. Importantly, stroking the artificially reared pups appears to reverse both the cognitive and the maternal deficits associated with artificial rearing (Lovic & Fleming, 2004). Moreover, the cognitive effects observed by Fleming and colleagues parallel closely with those reported as a function of differential maternal care over the first week of life [e.g., (T.Y. Zhang, Chretien, Meaney, & Gratton, 2005)].

Together, these data provide direct evidence that tactile stimulation in rodents during the postnatal period can have long-lasting behavioral sequelae in multiple behavioral domains such as cognition, affiliative behavior, and stress reactivity. These findings are consistent with Harlow and Zimmermann's classic work in macaque monkeys showing that tactile contact with an inanimate surrogate mother plays an important role in the emotional development and fearful response of the offspring (Harlow & Zimmermann, 1959). In humans, John Dieter and colleagues reported that massage therapy increases weight gain and alertness in preterm infants (Dieter, Field, Hernandez-Reif, Emory, & Redzepi, 2003), and Feldman et al. (2002) showed that premature babies provided with Skin-to-Skin Care during the first two weeks of life (i.e., wrapping the baby on the mother's chest for one hour daily) preformed better on cognitive and motor tasks at six months follow-up compared to the control group (Feldman, Eidelman, Sirota, & Weller, 2002).

The developing brain is exquisitely sensitive to sensory input

The ability of tactile stimulation provided by the dam to exert long-lasting effects on brain functioning is strikingly similar to the ability of other sensory inputs during the postnatal period to program brain development with sequelae that persist throughout the adult life of the animal. For example, amblyopia is a condition in which poor visual acuity occurs as a result of blocking one eye during early development

(Hensch, 2004). Similarly, exposure to specific tone frequencies during early development remodels the auditory cortex in rodents in a manner that also persists throughout life (Rubenstein & Merzenich, 2003). These paradigms appear to be conserved among rodents, primates, and humans, suggesting that they may share an important underlying principle of activity-dependent brain development. The conserved nature of these paradigms across different mammalian species supports the use of animal models to study the molecular mechanism by which sensory input shapes brain development in humans. Here we briefly describe details related to the ability of visual and auditory input early in life to modify brain development.

The monocular deprivation paradigm, initially described by Wiesel and Hubel (1963), is based on the observation that closure of one eye during a particular period in development leads to a permanent loss of visual acuity through that eye. The loss of visual acuity is due to reorganization of synaptic input that takes place in the visual cortex during the deprivation period. This synaptic reorganization occurs as a result of retracting synaptic input originating from the blocked eye followed by outgrowth of new axon terminals originating from the open eye to capture the available synaptic connections. For an excellent review on this issue see Hensch and Fagiolini (2005). Therefore lack of synaptic input from the deprived eye is responsible for the poor sight available from this eye. Importantly, reorganization of the visual cortex occurs only within a very specific developmental time frame (postnatal days 25–30 in rodents) known as the critical period and is not extended into adulthood. At the end of the critical period the system locks itself in a state that reflects available visual input during the critical period with no possibility for further reorganization. Reopening the blocked eye after the critical period will not restore synaptic input or visual acuity despite the fact that perfectly normal input is now available from this eye. Additionally, monocular deprivation (i.e., closure of one eye) in adulthood is not associated with reorganization of the visual cortex and therefore does not lead to changes in visual acuity from the deprived eye (Hensch, 2005). Similarly, work from Merzenich's group showed that following maturation of auditory pathways the rat's auditory cortex undergoes a remarkable reorganization that could be modified by exposure to auditory input provided during this period (Bao, Chang, Davis, Gobeske, & Merzenich, 2003; Chang & Merzenich, 2003; L.I. Zhang, Bao, & Merzenich, 2002). The primary auditory cortex develops as a two-dimensional map, known as a tonotopic map, with different zones within this map responding to a specific tone frequency referred to as their characteristic frequency. During the auditory critical period (postnatal days 14–35) this tonotopic map is refined in several ways: it shrinks in overall size, it develops areas that respond to lower frequencies not detected efficiently earlier, and the sensitivity and selectivity in which each zone responds to its characteristic tone frequency are improved (Nakahara, Zhang, & Merzenich, 2004). Interestingly, exposure of pups to specific frequencies during this critical period of tonotopic rearrangement leads to increased representations of these frequencies in the auditory cortex, alterations that are also maintained in adulthood. Importantly, exposure to the same frequencies does not alter the dam's tonotopic map, suggesting that this process of cortical organization is restricted to a specific developmental time frame (L.I. Zhang, Bao, & Merzenich, 2001).

In summary, at least some of the sequelae of handling are probably mediated by its ability to increase tactile stimulation provided by the dam during the first week of life. During this period the developing brain is capable of integrating sensory input into circuits that control fear and stress-induced HPA activation. Similarly, visual and auditory input during specific periods in early development are also capable of affecting brain functioning in a manner that persists into adulthood, suggesting that the developmental trajectory of the postnatal mammalian brain is modified in an activity-dependent manner by sensory input. The next section is concerned with how sensory input affects circuit assembly and circuit output during the postnatal period and how these changes are maintained throughout life. We begin by examining our current understanding of these issues in the monocular deprivation system, where important details concerning these questions have been characterized. We then discuss how tactile stimulation provided by the dam might affect neurodevelopment in a manner that alters behavior throughout life.

Part II: Long-term sequelae of postnatal sensory input

The extracellular matrix plays an important role in maintaining the long-term sequelae of monocular deprivation

Work from Michael Striker, Takao Hensch and others shows that the shift in synaptic reorganization of the visual cortex observed after monocular deprivation requires the maturation of specific cellular machinery capable of detecting competition between the two eyes (Hensch & Fagiolini, 2005). This machinery is composed of GABA-containing parvalbumin-positive cells that proliferate and form synapses with pyramidal cells in the visual cortex during the critical period. Using transgenic animals, Hensch's group showed that conditions that inhibit maturation and secretion of GABA by these cells also prevent the synaptic changes associated with monocular deprivation. For example, monocular deprivation fails to elicit synaptic remodeling in the visual

cortex of GAD65 knockout mice in which GABA synthesis is diminished (Hensch et al., 1998). However, local infusion of GABA agonists into the visual cortex of a GAD65 knockout mouse restored the effect of monocular deprivation on synaptic remodeling. Interestingly, administration of GABA agonist in adult GAD65 knockout mice was also associated with a synaptic response to monocular deprivation (Hensch et al., 1998). Additionally, adequate levels of brain derived neurotrophic factor (BDNF) are necessary for proliferation and maturation of parvalbumin cells (Jones, Farinas, Backus, & Reichardt, 1994). Conditions that elevate levels of BDNF early in development are associated with earlier maturation of parvalbumin cells and earlier onset of the critical period while conditions that decrease BDNF levels delay the onset of this period (Hanover, Huang, Tonegawa, & Stryker, 1999; Huang et al., 1999). Changes in levels of BDNF and other neurotrophic factors have been associated with onset of several other critical periods including variations in early maternal care (Bredy, Grant, Champagne, & Meaney, 2003; Liu, Diorio, Day, Francis, & Meaney, 2000). However, the exact contribution of neurotrophic factors to behavioral sequelae of maternal care has not been rigorously evaluated yet. We will return to this issue when discussing a possible mechanism by which maternal care can affect such a diverse array of behaviors (see Table 1 for details).

These and other data not presented here suggest that appropriate levels of BDNF are necessary for the proliferation and differentiation of parvalbumin-positive cells that in turn provide a surge of GABA input necessary for detecting competition between the two eyes and the synaptic reorganization associated with monocular deprivation. The exact mechanism by which GABA tone provided by parvalbumin cells helps to monitor competition between the two eyes is currently unclear (Hensch & Fagiolini, 2005). During the last days of the critical period, parvalbumin-positive cells are preferentially enwrapped with extra cellular matrix (ECM) to form perineuronal nets (Pizzorusso et al., 2002). Conditions that delay the critical period for monocular deprivation also delay formation of perineuronal nets (Pizzorusso et al., 2002). Moreover, administration of enzymes that degrade ECM in adulthood leads to restoration of neuroplasticity indistinguishable from that observed early in life, suggesting that the structural integrity associated with the formation of perineuronal nets and ECM deposition somehow 'locks' the system into its current state and prevents any additional changes in visually-mediated synaptic input (Pizzorusso et al., 2002). Several mechanisms have been postulated to account for the ability of ECM to restrict plasticity in the adult brain (Hensch & Fagiolini, 2005). First, it appears that certain proteoglycans found in the ECM provide a strong inhibitory signal for axon sprouting necessary for the synaptic remodeling of monocular deprivation. Second, the ECM might modify the ionic composition or other components of the extracellular milieu found around parvalbumin-positive cells, rendering them somehow unable to detect competition between the two eyes. Note that these two models are not mutually exclusive and that both may contribute to the ability of ECM to inhibit synaptic reorganization in the visual cortex. Regardless of the mechanism by which ECM deposition terminates the critical period, it is important to recognize that its stable presence throughout adulthood plays a critical role in maintaining the long-term sequelae of monocular deprivation early in life.

As discussed below, recent work has shown that DNA methylation appears to play an important role in maintaining the long-term effects of maternal care on gene expression and adult behavior (Weaver, Diorio, Seckl, Szyf, & Meaney, 2004). Together, these findings suggest the presence of several mechanisms by which sequelae of sensory input during early life could be maintained throughout life. Future work should examine whether DNA methylation established early in life may play a role in regulating genes that modify ECM properties relevant for neuroplasticity, and whether ECM plays also a role in maintaining some of the behavioral sequelae of postnatal maternal care.

Maternal care alters gene expression in the developing rodent brain in a manner that persists into adulthood

The blunted HPA reactivity observed in handled animals led to the search for genes that control HPA reactivity and whose expression is also modified by handling. One of the first genes to satisfy these two conditions was the glucocorticoid receptor (GR). Early work revealed that handling is associated with robust upregulation of GR expression in the hippocampus and prefrontal cortex in a manner that persists throughout the adult life of the animal (Meaney & Aitken, 1985; Meaney et al., 1989). Moreover, several lines of work indicate that levels of GR in the hippocampus and prefrontal cortex can modify HPA reactivity by yet undefined polysynaptic inhibitory tone on cells containing corticotropin-releasing hormone (CRH) located in the paraventricular nucleus of the hypothalamus (PVNh; please see Figure 1C for details related to the mechanism of HPA activation). Data to support the role of forebrain GR in maintaining an inhibitory control on HPA activity comes from several lines of work (de Kloet, 2000; Meaney et al., 1989; Sapolsky, Krey, & McEwen, 1984); however, the most compelling evidence is based on data showing that direct administration of corticosterone into the prefrontal cortex blunts stress-induced HPA reactivity (Diorio, Viau, & Meaney, 1993) and that a conditional knockout mouse in which expression of GR in forebrain is eliminated in adulthood shows increased corticosterone levels and

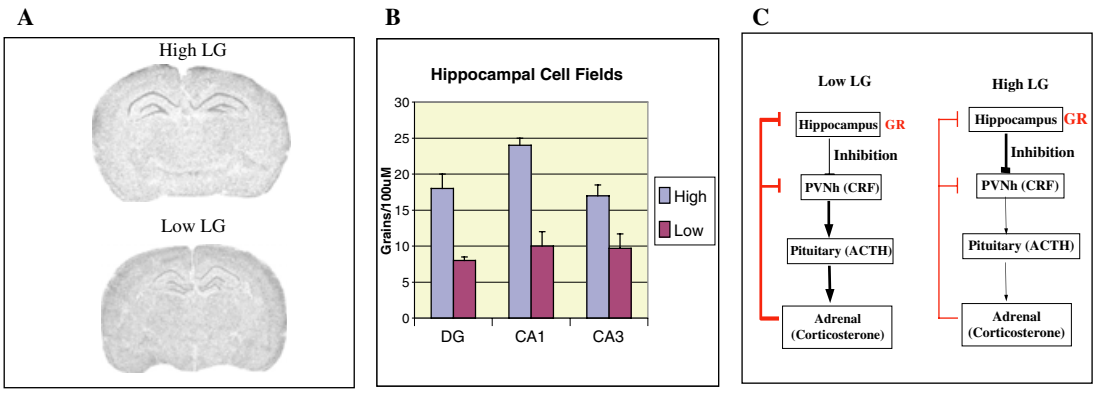

Figure 1 LG increases expression of GR in the hippocampus and decreases HPA reactivity. A. In-situ hybridization showing increase in GR mRNA in the hippocampus of animals exposed to high LG-ABN early in life. B. Quantification of GR mRNA expression in different subregions of the hippocampus as shown in A. C. Increased GR expression in the hippocampus of offspring raised by high LG-ABN dams is associated with increased inhibitory tone on cells in the paraventricular nucleus of the hypothalamus (PVNh) and therefore the blunted HPA reactivity in these animals (shown as thinner lines). DG: dentate gyrus, ACTH: adrenocorticotropin hormone, CRF: corticotrophin releasing factor, HPA: hypothalamic-pituitary-axis

increased vulnerability to stress (Boyle et al., 2005). Based on these observations, we proposed that increased levels of GR in forebrain of handled animals provide a more efficient inhibition of HPA activity (Viau, Sharma, Plotsky, & Meaney, 1993) and therefore the observed blunting of the HPA reactivity observed in handled animals (Meaney et al., 1989). Consistent with this hypothesis we have shown that conditions that down-regulate levels of GR in handled animals, such as seen after chronic administration of corticosterone, abolish the differences in HPA reactivity between handled and non-handled animals (Meaney et al., 1989).

Exposure to different levels of maternal LG during the postnatal period is also associated with HPA blunting and changes in forebrain GR levels that persist into adulthood (see Table 1 and Figure 1). As adults, offspring raised by high LG-ABN dams show increased levels of GR in the hippocampus as compared to offspring raised by low LG-ABN dams (Liu et al., 1997; Weaver, Cervoni et al., 2004; Weaver et al., 2005). Using a cross-fostering design we showed that pups born to low LG-ABN dams but raised by high LG-ABN dams were indistinguishable in terms of HPA reactivity and GR levels from the biological offspring of high LG-ABN dams, but were significantly different compared to littermates raised by low LG-ABN. Similarly, pups born to high LG-ABN but raised by low LG-ABN dams were indistinguishable in terms of stress reactivity and GR levels compared to the biological offspring of low LG-ABN dams (Weaver, Cervoni et al., 2004). These data demonstrate that postnatal maternal care is able to program GR expression levels in brain regions that control HPA activation in a manner that is stable throughout the adult life of the animal.

Most of the work to date has focused on the molecular mechanisms by which handling induces upregulation of GR levels in the hippocampus, with more recent studies assessing whether similar changes are also observed in response to variations in maternal care (i.e., frequency of LG-ABN). Here we provide a very brief description of the molecular details related to how handling in the first weeks of life influences transcription of GR in the hippocampus (for more details related to this work please see: Weaver, Diorio et al., 2004). The first physiological change associated with handling appears to be elevation in circulating levels of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). Initially, it was thought that the increase in thyroid hormones is an adaptive mechanism that protects the pup against hypothermia associated with its removal from the nest. However, we currently cannot exclude the possibility that changes in thyroid levels are due to increased LG-ABN frequency associated with handling. Recent study suggests the latter explanation. The day 3 offspring of high LG-ABN mothers show increased plasma T3 levels compared to those of low LG-ABN dams. Interestingly, plasma T4 levels show the opposite pattern (Hellstrom and Meaney, unpublished results). Since T3 is the biologically active variant derived from T4, this finding suggests that exposure to high LG-ABN enhances thyroid-induced metabolism due to deiodination of T4. Alterations in thyroid hormones appear to play an important role in inducing changes in GR levels. Administration of either T3 or T4 to pups on postnatal days 1, 2 and 4 is associated with increased GR levels in the hippocampus but not in the hypothalamus or pituitary of adult animals (Meaney, Aitken, & Sapolsky, 1987). Moreover, administration of the thyroid synthesis blocker propylthiouracil (PTU) during the first two weeks of life abolishes the ability of handling to upregulate expression of GR in the hippocampus (Meaney et al., 1987). These results demonstrate that an increase in circulating thyroid

levels is a necessary step for handling to elevate GR expression.

The effect of thyroid hormones on GR expression appears to be mediated by the ability of thyroid hormones to increase serotonin (5-HT) turnover and to chronically activate cyclic AMP mediated pathways in hippocampal cells through a 5-HT7 receptor (Laplante, Diorio, & Meaney, 2002; Mitchell, Betito, Rowe, Boksa, & Meaney, 1992; Mitchell, Iny, & Meaney, 1990). Interestingly, the effects of thyroid hormone administration on hippocampal GR levels are completely blocked with a 5-HT2/7 receptor blocker. Elevation in cAMP signaling is associated with increased expression of the transcription factor NGFI-A, which in turn binds to promoter elements on the GR DNA sequence to activate transcription in animals that have been handled (Meaney et al., 2000). Together, these observations suggest that handling leads to increased expression levels of the transcription factor NGFI-A that in turn supports a more efficient DNA binding of NGFI-A to the GR promoter and higher levels of GR expression in the hippocampus. Direct evidence that handling modifies NGFI-A binding to the GR promoter in vivo is not currently available. However, chromatin immunoprecipitation experiments show that in vivo, NGFI-A binding to GR promoter in adult animals raised by high LG-ABN dams is dramatically increased compared to animals raised by low LG-ABN dams (Weaver, Cervoni et al., 2004). Importantly, studies with primary hippocampal cell cultures show that GR expression is increased following treatment with stable cAMP analogs or by 5-HT7 receptor agonists; these effects are completely blocked with an antisense directed against NGFI-A mRNA (Weaver et al., submitted).

Unlike the postnatal period where high LG-ABN is associated with elevated levels of NGFI-A, no differences in NGFI-A levels were observed in adult animals raised by low and high LG-ABN dams (I.C.G. Weaver, J. Diorio, & M.J. Meaney, unpublished results). This observation raised the following questions: First, given comparable levels of NGFI-A in the adult hippocampus, what is responsible for the observed difference in NGFI-A ability to bind and activate transcription of GR later in life? Second, what is the mechanism by which maternal care in the first weeks of life maintains differential DNA binding of NGFI-A in the adult animals (and therefore regulates gene expression), long after the pups have been weaned and separated from the dam? The answer to both questions appears to be the same and is currently thought to be due to the ability of maternal care to modify DNA methylation during the first week of life. DNA methylation is composed of a carbon–carbon bond that is very stable. Thus DNA methylation provides a stable 'molecular fence' that decorates the DNA and regulates gene expression in neurons, and other post-mitotic cells, for the rest of the animal's life, unless very special circumstances are used to modify it (see also below). Moreover, as explained in details below, DNA methylation can also modify the affinity of transcription factors for their binding sites. Indeed, DNA methylation is commonly thought to inhibit transcription factor binding to DNA and thus silence gene expression (Bird & Wolffe, 1999). Thus, the ability of maternal care to modify DNA methylation during a critical period in development provides a possible explanation as to how maternal care early in life influences DNA binding and transcriptional regulation in adulthood.

Introduction to DNA methylation

DNA in cells is arranged in a complex three-dimensional structure composed of DNA, histone proteins, and RNA known as chromatin (for detailed reviews see Hsieh & Gage, 2004; Jaenisch & Bird, 2003; Lund & van Lohuizen, 2004). Chromatin allows cells to condense the long linear DNA molecule into a more compact three-dimensional structure that fits into the cell nucleus, while at the same time maintaining specific regions of the DNA either accessible or 'closed off' (i.e., silenced) for transcriptional activation based on environmental and developmental needs. The first step of DNA packing involves wrapping DNA around a positively charged histone octomer core composed of four histone subunits (histone 2a, 2b, 3 and 4; histone 1 serves as a link between nucleosomes). Since the DNA backbone is negatively charged, it wraps tightly around the positively charged histone core forming a string of yo-yo-like structures (i.e., nucleosomes) that are connected via segments of linear naked DNA. Proteins and RNA bind to this partially folded chromatin and further modify it to form a more compact structure. This compact and complex structure provides a cellular solution to the problem of fitting a long linear molecule such as DNA into a small nucleus (imagine compressing 20 km of thread into a grapefruit), but creates a new problem accessing specific sites on the DNA to activate gene expression. To solve this problem, cells developed a complex machinery that is capable of opening and closing chromatin in a manner that provides access to some regions while maintaining others packed and inaccessible. One such mechanism involves the recruitment of a specialized class of proteins known as histone acetyltransferases (HAT). These proteins are capable of adding an acetyl group to positively charged amino acids on specific histones. This in turn reduces the affinity of the now less positively charged histone core to the negatively charged DNA, making it now more accessible for transcription. On the other hand, a rival group of proteins known as histone deacetylases (HDAC) can remove the acetyl group placed by HAT and render the chromatin less accessible again. Therefore, recruitment of HAT or HDAC to a specific location within the DNA plays an important role in DNA accessibility and gene

expression. In most cases, when DNA is methylated it recruits methylated DNA binding proteins that attract a repressor complex that includes HDAC and therefore silences gene expression.

DNA methylation involves a family of enzymes known as DNA methyltransferases (DNMT). These proteins scan the DNA and transfer a methyl group from a methyl donor molecule such as S-adenosyl-methionine to a cytosine ring found in the dinucleotide sequence CpG. As mentioned above, the addition of a methyl group to the 5′ cytosine ring forms a high-energy carbon-carbon bond that is very stable. Methylation of CpG then recruits HDAC that as described above further compacts chromatin structure in the vicinity of these methylated sites rendering them less accessible for gene expression. This form of transcriptional regulation is referred to as epigenetic regulation. Unlike genetic regulation of gene expression that involves alteration of a DNA sequence, epigenetic regulation can modify transcription from an identical DNA sequence and therefore plays an important role in tissue differentiation and cancer biology. (For details see: Jaenisch & Bird, 2003; Szyf, Weaver, Champagne, Diorio, & Meaney, 2005.) Moreover, the relative stability of DNA methylation makes it an ideal mechanism in post-mitotic cells to maintain long-lasting changes in gene expression. Importantly, most of the work to date on DNA methylation has been done in the context of cancer biology and early development (e.g., gene imprinting) in which DNA methylation and demethylation occur in mitotically active cells (i.e., dividing cells). Recent findings from several labs, including findings described below, have now documented changes in DNA methylation in post-mitotic cells (Bruniquel & Schwartz, 2003; Cervoni, Detich, Seo, Chakravarti, & Szyf, 2002; Cervoni & Szyf, 2001; Detich, Theberge, & Szyf, 2002; Martinowich et al., 2003). However, the role of DNA methylation in post-mitotic cells is still quite controversial, and more work is needed to replicate some of the results and to further characterize the molecular machinery involved. However, if our proposal that DNA methylation does play a role in maintaining the long-term sequelae of postnatal maternal care gains further support, it would open a new horizon in developmental cell biology with important implications for treatment and diagnosis of psychopathology associated with early life stress (see also below).

The role of DNA methylation in maintaining behavioral sequelae of postnatal maternal care

A longitudinal assessment of the methylation state of the GR promoter during the postnatal period reveals that very shortly after birth, this promoter is hypermethylated on many CpG sites, including two sites that are localized within the DNA binding site for NGFI-A (Weaver, Cervoni et al., 2004). Exposure to high LG-ABN during the following five days of life is associated with a dramatic reduction in DNA methylation at the GR promoter, which then persists throughout the adult life of the animal (Weaver, Cervoni et al., 2004). See also Figure 2. Using a gel-shift assay we showed that methylation of the NGFI-A binding site that is similar to the one observed in vivo reduces the affinity of NGFI-A to its binding site in vitro (Weaver, Diorio et al., 2004). This is consistent with the chromatin immunoprecipitation studies showing that NGFI-A binds more efficiently in vivo to GR promoter of adult animals raised by high LG-ABN (i.e., where the promoter is hypomethylated) as compared to those raised by low LG-ABN (Weaver, Cervoni et al., 2004). Indeed, despite comparable levels of NGFI-A, there is about 3–4-fold greater NGFI-A binding to the Pl_7-GR promoter in the adult offspring of high compared with low LG-ABN mothers. Thus, high levels of maternal LG-ABN during the first week of life are associated with promoter remodeling that allows NGFI-A to bind its site and

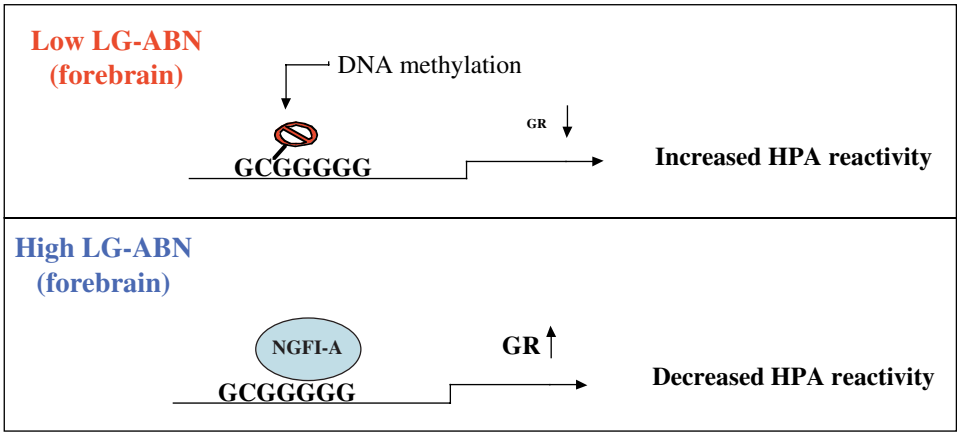

Figure 2 DNA methylation pattern established early in life is responsible for the ability of postnatal maternal care to regulate expression levels of GR and HPA reactivity in a manner that persists into adulthood. Exposure to high levels of LG-ABN is associated with demethylation of the GR promoter allowing the transcription factor NGFI-A to bind and activate expression of GR in the hippocampus. The stability of such DNA modifications provides a molecular mechanism to explain how events early in life shape behavior in adulthood.

support a more efficient transcription of GR in the hippocampus. Conversely, pups exposed to low levels of LG-ABN maintain the GR promoter in its hypermethylated state, which in turn inhibits binding and reduces transcriptional activation of GR by NGFI-A (Weaver, Cervoni et al., 2004). To test whether this change in methylation is due to postnatal maternal care or to a possible difference in prenatal maternal care between high and low LG-ABN dams, we cross-fostered pups born to low LG-ABN dams into litters of high LG-ABN dams and pups born to high LG-ABN dams were raised by low LG-ABN dams. These experiments demonstrated that the GR-Pl_7 promoter of pups raised by high LG-ABN is demethylated, while those raised by low LG-ABN were hypermethylated regardless of the maternal phenotype of the biological mother (Weaver, Cervoni et al., 2004). These data provide an association between levels of LG-ABN during the first week of life and DNA methylation at the GR promoter. Moreover, once these patterns of promoter methylation are established, they persist into adulthood and therefore provide a possible molecular mechanism by which early maternal care could program GR expression and vulnerability to stress throughout life (Weaver, Cervoni et al., 2004). The developmental time frame of the GR promoter remodeling is consistent with the ability of handling during the first five days of life to alter stress reactivity in rodents (Hess et al., 1969; Meaney et al., 1989) and suggests that in rodents, the first week of life represents a critical period in which tactile stimulation provided by the dam is able to modify neurodevelopment of circuits that control stress reactivity in a manner that persists into adulthood.

To further assess the role of promoter methylation in regulating GR expression in adulthood we infused the HDAC inhibitor trichostatin A (TSA) intraventricularly into adult animals raised by high and low LG-ABN dams (Weaver, Cervoni et al., 2004). The rationale for this experiment is based on the hypothesis that demethylation can occur under conditions in which active demethylase is present and the chromatin structure is open and accessible for demethylation. If active demethylase is present in post-mitotic cells then HDAC inhibitors could trigger demethylation by making the chromatin structure more accessible. This hypothesis is consistent with tissue culture work from Moshe Szyf's lab showing that HDAC inhibitors such as TSA and valproate can demethylate DNA in a replication-independent manner (Cervoni et al., 2002; Cervoni & Szyf, 2001; Detich, Bovenzi, & Szyf, 2003; Detich et al., 2002). Indeed, administration of TSA into adult animals raised by low LG-ABN (i.e., those with hypermethylated GR promoter and poor NGFI-A binding) led to the expected increase in promoter acetylation, but was also associated with increased NGFI-A binding and decreased methylation. Importantly, this reversal of adult GR promoter methylation was associated with increased GR expression and decreased HPA reactivity in these animals making them indistinguishable from those raised by high LG-ABN. Moreover, TSA infusion to animals raised by high LG-ABN had no effect on DNA methylation, NGFI-A binding, GR expression and HPA reactivity (Weaver, Cervoni et al., 2004). Similarly, intraventricular infusions of methionine which acts as an intermediate donor for DNA methylation increased methylation of the GR promoter, decreased expression of GR, and increased HPA reactivity in animals raised by high LG-ABN (i.e., those with hypomethylated promoter) but had no effect on animals raised by low LG-ABN (Weaver et al., 2005). These data demonstrate that DNA methylation can be altered in post-mitotic cells and are consistent with the notion that DNA methylation in post-mitotic cells exists in a dynamic steady state that reflects a balance between rates of methylation and demethylation. The reader may well wonder if such broad pharmacological manipulations altered the expression of a wide range of genes in the hippocampus. Somewhat to our surprise this was not the case. Microarray analysis of hippocampal samples revealed that infusion of TSA or methionine changed the expression of less than 3% of the hippocampal 'transcriptome' [i.e., genes normally expressed in the hippocampus; (Weaver, Meaney, & Szyf, 2006)].

Recent work reveals that postnatal maternal care is also capable of remodeling the estrogen receptor alpha (ER-α) promoter in the medial preoptic area (MPOA) of the hypothalamus of female offspring, providing another example in which DNA methylation may contribute to maintaining the long-term sequelae of postnatal maternal care (F. A. Champagne et al., 2006). As adults, female offspring raised by high LG-ABN dams become high LG-ABN and those raised by low LG-ABN become low LG-ABN dams. Importantly, this maternal phenotype appears to be stable throughout the adult life of the animal and is also observed in the dam's behavior towards future litters (F. A. Champagne et al., 2003). Cross-fostering experiments demonstrated that maternal behavior in the offspring correlates with the maternal phenotype of the foster and not the biological mother, indicating that maternal phenotype in the offspring is strongly influenced by the frequency of LG-ABN the offspring received early in life (D. Francis, Diorio, Liu, & Meaney, 1999). Likewise, the female offspring of high LG-ABN mothers (regardless of biological origin) show increased ER-α expression in the MPOA. A wealth of neuroendocrine studies show that the expression of maternal behavior in the rat is dependent upon the activation of ER-α in the MPOA and downstream effects on oxytocin receptor binding (Numan & Sheehan, 1997; Pedersen, Caldwell, Walker, Ayers, & Mason, 1994). Female offspring raised by low LG-ABN dams show increased DNA methylation in a Stat5 regulatory element localized within exon 1b of the ER-α gene as compared

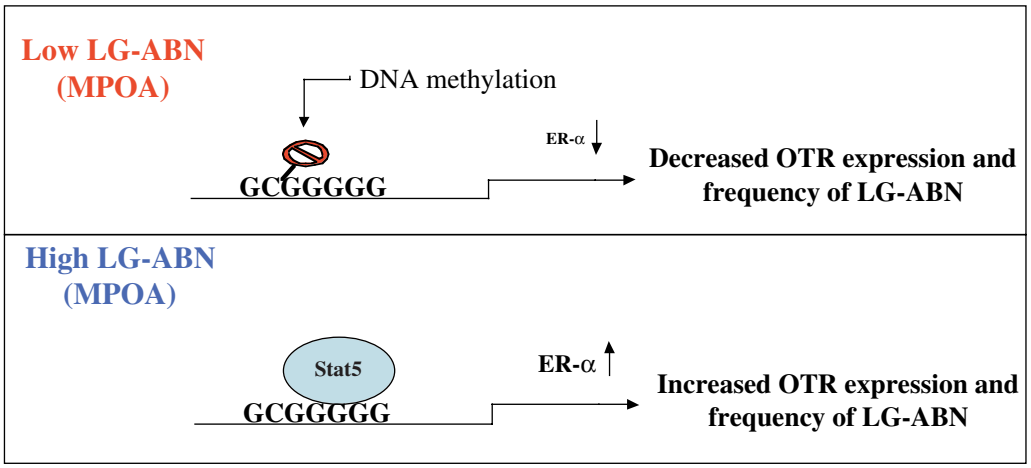

Figure 3 Frequency of LG-ABN in the first week of life modifies DNA methylation of the ER-α promoter that is thought to be responsible for a stable maternal phenotype in female offspring. Exposure to high levels of LG-ABN is associated with demethylation of the ER-α promoter allowing the transcription factor Stat5 to bind and activate expression of ER-α in the medial preoptic area (MPOA) of the hypothalamus. Elevated ER-α levels render female offspring of high LG-ABN dams more sensitive to elevated levels of estrogen associated with pregnancy and result in higher levels of postpartum oxytocin receptor (OTR) in the MPOA. Elevated levels of oxytocin receptor in the MPOA are in turn responsible for increased levels of LG-ABN in these offspring. The ability of maternal care early in life to 'program' a stable maternal phenotype in the female offspring ensures epigenetic transmission of maternal phenotype and many other behavioral phenotypes influenced by early maternal care across generations.

to those raised by high LG-ABN dams. These changes in methylation state are associated with decreased binding of the transcription factor Stat5 to its binding site, resulting in lower levels of ER-α in the MPOA of adult females raised by low LG-ABN (F. A. Champagne et al., 2006). MPOA levels of ER-α appear to control expression of oxytocin receptor (OTR) in the MPOA (Young, Wang, Donaldson, & Rissman, 1998), which in turn regulates several aspects of maternal behavior such as frequency of LG-ABN (See Figure 3). Data in support of this proposed model comes from studies showing that levels of OTR are elevated in offspring of high LG-ABN compared to low LG-ABN. Ovariectomy eliminates the difference in OTR levels between offspring of low and high dams, but these differences can be reinstated again after addition of exogenous estrogen, suggesting that differences in OTR levels require the presence of estrogen (F. Champagne, Diorio, Sharma, & Meaney, 2001). Additionally, direct administration of either estrogen (Fahrbach, Morrell, & Pfaff, 1985) or oxytocin (Pedersen et al., 1994) into the MPOA increases maternal behavior and central administration of OTR-antagonist on postnatal day 3 abolishes the differences in LG-ABN between high and low dams (F. Champagne et al., 2001). These observations suggest that activation of OTR in the MPOA is important in maintaining individual differences in LG-ABN. Thus the hypomethylated state of the ER-α promoter of offspring exposed to high frequency of LG-ABN early in life provides a more efficient transcriptional mechanism to upregulate OTR in response to increased estrogen levels associated with pregnancy. Elevated levels of OTR in the MPOA and probably other brain regions are then responsible for the increase in LG-ABN frequency observed in female offspring exposed to higher levels of maternal care in infancy, probably through subsequent regulation of dopamine release at the level of the nucleus accumbens (F. A. Champagne et al., 2004; Numan & Sheehan, 1997).

Several important caveats related to the possible role of DNA methylation in programming stable behavioral traits are important to consider. First, we cannot currently rule out the possibility that mechanisms other than changes in methylation of the GR promoter play a role in the ability of intraventricular infusions of TSA or methionine to modify HPA reactivity. However, regardless of the exact mechanism by which these interventions modify HPA reactivity, they show very specific interactions with early maternal care (i.e., TSA administration affects only offspring of low LG-ABN with no effect on those raised by high LG-ABN). Second, if DNA methylation is not as permanent as initially thought, how is it maintained throughout life and what conditions can modify it? For example, we currently do not know whether changes in DNA methylation, GR expression and HPA reactivity are stable or revert back to pretreatment condition in adult animals treated with TSA or methionine once these treatments have stopped. Moreover, high LG-ABN dams exposed to stress during pregnancy show decreased frequency of LG towards their pups and become indistinguishable from low LG-ABN dams (F.A. Champagne & Meaney, in press). It is currently unclear whether these behavioral changes in maternal phenotype are associated with reversal of ER promoter methylation or whether they represent a compensatory modification provided by the environment without revers-

ing the DNA methylation. These issues are important to consider when evaluating therapeutic interventions that seek to reverse or modify the effects of early development on adulthood and will be considered in more detail below. Finally, the pharmacological manipulations used to reverse the methylation status of the relevant GR promoter are indeed very crude. However, and this is the important consideration in such studies, these manipulations do reveal that the enzymatic machinery necessary to demethylate or even remethylate specific DNA sites exists in post-mitotic neurons, even in the adult phase of the life cycle.

Epigenetic transmission of maternal care in non-human primates

The ability of postnatal maternal care to program maternal behavior, vulnerability to stress, and cognition (see also below) in the offspring allows for an epigenetic mode of inheritance for these behavioral phenotypes. In other words, offspring of low LG-ABN dams are fearful and as adults lick their own pups infrequently, raising yet another generation of animals that are also fearful and low lickers in a pattern that propagates itself vertically across generations. Epigenetic modes of transmission differ from the more 'traditional' genetic transmission in two fundamental ways. First, epigenetic transmission is not mediated by alterations in DNA sequence, but by stable modifications of existing DNA sequences such as DNA methylation. Second, genetic transmission is passed across generations via the germline while epigenetic transmission described above is passed to the next generation via maternal care provided to the offspring early in life (the modifications to DNA methylation described above do not involve the germ-line). The relative stability of DNA methylation leads to a stable maternal phenotype in adulthood that ensures propagation of the maternal phenotype across generations. The historical irony of these findings is that they suggest that Jean Baptiste Lamarck might have been right when he proposed that acquired modifications could be transmitted across generations, a point lost due to the radical fervor of the 'neo-Lamarckians.'

The adaptive value of this kind of epigenetic mode of transmission is quite speculative at this point and here we consider two possible explanations. The first explanation argues that environmental challenges encountered early in development are likely to persist throughout life, and therefore neurodevelopmental programming that incorporates these environmental challenges may improve survival in adulthood. For example, the fearful phenotype induced by low LG might be protective for offspring raised under stressful environmental conditions associated with high levels of predation and scarce resources. The observation that daily restraints of high LG-ABN dams during the prenatal period are associated with a decrease in frequency of LG (F.A. Champagne & Meaney, in press) is consistent with this hypothesis. Additionally, work from Rosenblum and Coplan assessed the effect of postnatal environmental stress on mother-infant interactions and their behavioral sequelae in the Bonnet macaque offspring. Their initial paradigm has used three different postnatal conditions: 1) a low foraging condition in which food is readily available, 2) a high foraging condition in which adequate food supply was available but required long periods of foraging, and 3) a variable foraging demand condition in which the two conditions are randomly assigned making food availability unpredictable. Using this paradigm they showed that the variable foraging condition was the most disruptive of maternal care and was associated with increased CRF levels and abnormal fear response in the offspring (Coplan et al., 2005; Coplan, Rosenblum, & Gorman, 1995; Coplan et al., 1998; Rosenblum et al., 1994). These data demonstrate that environmental stress modifies maternal behavior and is associated with behavioral sequelae in the offspring, but it is yet to be shown that offspring raised under variable foraging conditions cope better with these conditions in adulthood as compared to the control group. An alternative hypothesis is that low levels of maternal care induce a catabolic 'spore like' state of neurodevelopment that is adaptive in several ways. First, the lower threshold for stress reactivity may increase the capacity of the pup to recruit additional parental care by emitting more frequent ultrasonic calls. Second, unrestricted growth and neurodevelopment in the presence of inadequate parental support may result in poorer cognitive and emotional outcomes associated with decreased potential for reproductive success in these offspring.

The ability of maternal care in rodents to epigenetically transmit several behavioral phenotypes across generations suggests the possibility that similar phenomena may exist in non-human primates and humans. The best evidence for epigenetic transmission of behavior in non-human primates comes from recent work by Dario Maestripieri on maternal abuse in macaque monkeys (Maestripieri, 2005). Infant abuse in the macaque occurs in the first few months of life and consists of dragging, throwing, sitting on, or biting the infant with consequences that range from minimal injuries to infant mortality (Maestripieri, 1998). Maternal abuse has been reported in both captive and free-ranging macaques (Maestripieri, 2005). Importantly, this maternal phenotype is stable across pregnancies, and runs in families with an apparent transmission of this behavior from mother to daughter (Maestripieri, 1999). Finally, intergenerational transmission of childhood abuse has also been described in humans and the prevalence of infant abuse in the macaque (5–10%) is comparable to the one estimated in humans (20–30%) (Maestripieri, 1999). Indeed, in humans

there is evidence for the intergenerational transmission of a wide range of parental styles (Miller, Kramer, Warner, Wickramaratne, & Weissman, 1997; Warner, Weissman, Mufson, & Wickramaratne, 1999).

To determine whether the familial pattern of maternal abuse in the macaque is due to genetic or epigenetic transmission, Maestripieri used a cross-fostering paradigm in which offspring born to non-abusive mothers were cross-fostered soon after birth to abusive mothers (control/abuse) and infants born to abusive mothers were cross fostered to non-abusive mothers (abuse/control). These two groups were also compared to two additional groups of abusive and non-abusive mothers raising their own infants (abuse/abuse and control/control respectively). The maternal phenotype of the adult offspring was then evaluated after they gave birth to their first offspring. The results showed that 9 of the total 16 females that were abused in childhood became abusive as adults while none of the mothers raised by non-abusive mothers (abuse/control and control/control) became abusive. The striking result was that the rates of childhood abuse were similar between the control/abuse and abuse/abuse groups (four out of eight and 5 out of eight respectively) and these were significantly different from offspring raised by non-abusive mothers regardless of their biological mother's phenotype (Maestripieri, 2005). These results demonstrate that exposure to abusive mothers postnatally is associated with a significant risk for future childhood abuse regardless of the biological mother and strongly suggests that transmission of infant abuse in the macaque is mediated by an as yet undefined epigenetic mechanism. We suggest that, as in the case of rodent licking/grooming, the individual differences in maternal care may be transmitted across generations through a behavioral mode of transmission.

Work from several groups using different paradigms demonstrates that postnatal maternal care in non-human primates plays an important role in shaping fear responses, social play, aggression, and vulnerability to substance abuse in the offspring. For excellent reviews see: (Barr et al., 2003; Coplan et al., 1995). Together with Maestripieri's work one cannot ignore the similarities between findings in rodents and non-human primates with regard to the effect of early-life care on diverse behavioral phenotypes. The observations that maternal deprivation in early-life increases HPA reactivity in non-human primates (Barr et al., 2004; Rosenblum et al., 1994), and that women exposed to early-life stress show enhanced HPA activation under stress (Heim et al., 2000) suggest that alterations in early-life care may lead to changes in neurodevelopment that are conserved among mammals. Moreover, despite differences in postnatal brain development between rodents, non-human primates, and humans, they all follow remarkably similar neurodevelopmental trajectories in response to sensory input during a critical period of development. For example, unlike primates that are born with rudimentary visual acuity, rodents are born with their eyes closed and have no visual sensory input during the first week of life. Despite these differences both respond to monocular deprivation in a very similar way, with the principal difference being the exact developmental period that monocular deprivation affects vision in rodents and humans [PND 25–30 in mice and approximately 1–4 years in humans (Hensch, 2004)]. These observations suggest a possible shift in the developmental time frame between rodents and primates, but at the same time they underscore a remarkable conservation of developmental principles between the two species. Moreover, some of the most significant progress in our understanding of the molecular details that guide monocular deprivation has been through the use of transgenic animals by Takao Hensch's group (Fagiolini et al., 2004; Hensch et al., 1998; Mataga, Mizuguchi, & Hensch, 2004).

Part III: Epigenetic mode of inheritance: implications and complexity

Epigenetic transmission in humans: research implications

Epigenetic transmission in humans is difficult to evaluate because most adoption studies have not controlled for time of adoption and the critical period for most phenotypes is not known. Therefore interpretation of most adoption studies is tainted by the possibility that the adoption occurred after a possible period during which relevant developmental events occur. However, findings in rodents (Denenberg & Rosenberg, 1967; D. Francis et al., 1999) and non-human primates (Maestripieri, 2005) strongly suggest that epigenetic transmission of behavioral traits is also likely to be found in humans. An important clinical and research implication of this possibility is that several psychopathologies that run in families such as depression, anxiety (Mullen et al., 1996), and possibly some forms of schizophrenia (Bebbington et al., 2004) might be transmitted via genetic and/or epigenetic modes of inheritance. The point here is certainly not to contest the potential importance of classical genetic variation (i.e., variations in sequence that are inherited from parents), but to simply underscore the possibility that such effects occur in parallel and probably interacts with environmentally-driven epigenetic modifications to the genome.

Current genetic research focuses exclusively on identifying genetic mechanisms responsible for this mode of transmission. This approach, however, has yielded relatively few examples of genes that are reproducibly associated with psychopathology in humans. Several reasons have been mentioned to explain the difficulty in mapping 'psychiatric genes' (Kendler, Gardner, & Prescott, 2006; Moffitt, Caspi,

& Rutter, 2005). These include: the heterogeneity of the phenotype (i.e., depression), the lack of objective measurements to assess the phenotype, and the complex polygenetic nature of these disorders. What has been missing in this important list of complexities is the possibility that epigenetic transmission may play an important role in propagating some of these psychopathologies within families. Indeed, gene–environment interactions that form the logical basis for epigenetics have only recently been investigated (Mill et al., 2006). The results of these studies strongly suggest that the effects of genetic variations depend upon environmental conditions (and vice versa). Hence linkage analysis studies should not be considered as adequate tests of the potential relevance of variations in genotype for any specific phenotypic outcome. Inevitably, the potential relevance of any genomic variation in sequence or any specific environmental event will only be fully understood within a gene–environment framework. This framework should include epigenetic modifications. This is especially important given the preclinical work presented above and strong clinical data demonstrating an association between early-life stress and increased risk for several psychopathologies in humans (Moffitt et al., 2005). It is important to acknowledge that the tissue-specific nature of epigenetic modifications makes the search for such alterations more difficult compared to the traditional search for genetic factors. For example, methylation of the GR promoter seen in the hippocampus may not be present in peripheral tissues available to assess promoter methylation (i.e., obtaining human brain tissue for diagnostic purposes is not an option). On the other hand, since all cells in our bodies contain the same DNA sequence, mutations seen in peripheral blood cells will also appear in the hippocampus. The tissue-specific nature of epigenetic modifications clearly represents a challenge for future research; however, given its potential for elucidating an important mechanism by which the environment modifies gene expression, such an effort is likely to be both informative and productive.

Gene–environment interactions

Work by Avshalom Caspi and his colleagues provides compelling evidence that both genetic predisposition and environmental exposure need to be considered in assessing several behavioral traits and vulnerabilities for psychopathologies (Moffitt et al., 2005). For example, Caspi et al. (2003) showed that individuals that are homozygous for the short allele of the serotonin transporter exposed to early-life stress are more likely to develop depression in adulthood as compared to individuals with the more 'protective' long allele of the serotonin transporter (5-HTT) (Caspi et al., 2003). Importantly, these findings have now been replicated by several other groups and have been extended to children and adolescents (Eley et al., 2004; Kaufman et al., 2006). 5-HTT polymorphism also mediates vulnerability to stress in maternally deprived monkeys (Barr et al., 2004), and knockout mice with a deletion in the 5-HTT show increased fearful behavior in a dose-dependent manner (Holmes, Lit, Murphy, Gold, & Crawley, 2003) (i.e., homozygous knockout mice are more fearful than heterozygote, which are more fearful than wildtype littermates) that is most likely due to low levels of the 5-HTT early in life (Ansorge, Zhou, Lira, Hen, & Gingrich, 2004). These observations suggest that the developmental effects of gene–environment interactions are conserved between rodents, primates, and humans, and can be further studied in such animal models (Holmes, Murphy, & Crawley, 2003; Meaney, 2001). Despite the well-accepted notion that both susceptibility genes and postnatal parental care play important roles in programming vulnerability to stress, the details of how their interactions shape stress reactivity are currently unknown. For historical reasons, most of the work to date has characterized the effects of postnatal maternal care (or early-life stress) on neurodevelopment in rats with very few studies reported in the mouse, and no group has yet systematically used transgenic animals to study this problem. As a result, we know much about how early maternal care influences brain development in the outbred Long-Evans hooded rats, but have no information on how this process interacts with different genetic backgrounds. To address this issue we and other groups have adopted a paradigm established initially by Hymie Anisman, which uses two strains of inbred mice, C57 and Balbc, that differ with regard to their level of maternal care and reactivity to stress. C57 dams lick their pups almost twice as much as compared to Balbc and their offspring are less fearful compared to Balbc (Anisman, Zaharia, Meaney, & Merali, 1998; Caldji, Diorio, Anisman, & Meaney, 2004; D. D. Francis, Szegda, Campbell, Martin, & Insel, 2003). Balbc offspring raised by the high LG C57 dams are less fearful and perform better in the visual-spatial task of the Morris water maze as compared to those raised by the low LG Balbc dams (Caldji et al., 2004; Zaharia, Kulczycki, Shanks, Meaney, & Anisman, 1996). Interestingly, behavior of C57 offspring shows very little change in response to cross fostering, suggesting that they might be more resilient (or less responsive) to the neurodevelopmental effects of cross fostering (Caldji et al., 2004; Zaharia et al., 1996), Kaffman & Meaney, unpublished work).

To further assess whether the observed resiliency of C57 pups is due to differences in genetic background (i.e., genotypes of C57 versus Balbc pups) postnatal maternal care or environment (i.e., in utero differences between Balbc and C57 dams), Francis et al. transplanted C57 embryos into pseudo-pregnant Balbc dams and assessed the effect of raising these pups by the high LG C57 or the low LG Balbc

dams (D. D. Francis et al., 2003). This created four experimental groups of C57 mice: 1) those raised prenatally and postnatally by C57, 2) those raised prenatally by C57 and postnatally by Balbc, 3) those raised prenatally by Balbc and postnatally by C57, and 4) those raised prenatally and postnatally by Balbc. Of these four groups only C57 embryos transplanted into Balbc and raised postnatally by Balbc (group 4) resembled Balbc offspring in most but not all behaviors tested. These elegant experiments suggested that genetic background, prenatal conditions, and postnatal maternal care all play a role in establishing individual differences. It is our assessment that the ability to manipulate genes, maternal care, and exposure to stress makes the mouse a unique experimental system to understand the molecular details of how gene and early environment interactions affect neurodevelopment. Establishing a robust and reliable experimental system in the mouse to study this process represents an important task for future efforts.

Environmental enrichment can improve cognitive performance of offspring raised by low LG-ABN dams

In previous sections we presented data showing that several pharmacological interventions provided in adulthood modified the methylation state of the GR and HPA reactivity in these animals. We also mentioned that the stability of these changes once the medication has stopped is currently unknown. Here we discuss data showing that cognitive performance associated with early-life care could be modified by a non-pharmacological intervention provided from postnatal day 22–70 (i.e., prepubescence into adulthood) (Bredy, Humpartzoomian, Cain, & Meaney, 2003). Offspring of high LG-ABN dams perform better in several hippocampal-dependent memory tasks compared to offspring of low LG-ABN (Liu et al., 2000). Cognitive performance in these tasks is dependent on changes in hippocampal neuroplasticity that are mediated by two families of receptors: the N-methyl-D-aspartate (NMDA) and -amino-3-hydroxy-5-methyl-4-isox-azolepropionic acid receptor (AMPA). NMDA and AMPA receptors induce long-lasting electrophysiological changes in synapses within the hippocampus known as long-term potentiation (LTP) and long-term depression (LTD) that are required for appropriate learning and memory (Bailey, Bartsch, & Kandel, 1996; Bailey, Kandel, & Si, 2004; Bliss & Collingridge, 1993; Tang et al., 1999). Offspring of high LG-ABN dams show increased levels of NR1, NR2A, and NR2B subunits of the NMDA receptors in the hippocampus and enhanced LTP as compared to offspring of low LG-ABN. These findings suggested that maternal care improves learning by enhancing NMDA-mediated neuroplasticity in the hippocampus. Exposure of low LG-ABN prepubescent offspring (PND22–70) to environmental enrichment improves their performance in several hippocampal cognitive tasks, making them indistinguishable from offspring of high LG-ABN dams (Bredy, Humpartzoomian et al., 2003). Note that performance of offspring raised by high LG-ABN dams is not improved further by environmental enrichment. Interestingly, environmental enrichment increases levels of AMPA receptor in offspring of low LG-ABN without affecting NMDA levels or LTP (Bredy, Humpartzoomian et al., 2003). These data demonstrate that the functional consequences of postnatal maternal care (i.e., hippocampal-dependent cognition) can be altered by additional environmental input later in life, and that the mechanism by which environmental enrichment improves cognition does not appear to reverse changes established early in life (i.e. low levels of NMDA receptors), but rather to modify a compensatory alternative pathway (i.e., increase in AMPA receptors). These findings are but one example of the potential for environmentally-induced alterations of phenotype at later stages in development.

Active parenting and resiliency

An intriguing implication of the rodent work is that it suggests that active parenting is required to modify neurodevelopment and induce resiliency to stress. Note that low levels of maternal care simply maintain a default state of promoter hypermethylation established at birth, while high levels of maternal care are required to demethylate the GR promoter and therefore presumably increase resiliency to stress during life (Weaver, Cervoni et al., 2004)(see Figure 2). If similar processes also occur in humans, these data suggest that interventions that seek only to eliminate neglect and abuse in maltreated children, though obviously necessary and important, may not be sufficient to promote resiliency in these children. Thus, future work may need to examine more closely how parental care enhances resiliency in non-human primates and children instead of focusing almost exclusively on how childhood abuse and neglect enhance psychopathology. This is consistent with data showing that poor parental bonding, not considered abuse or neglect, is also associated with a significant increased risk for depression and anxiety disorders (Canetti et al., 1997) as well as with enhanced stress reactivity (Luecken & Lemery 2004; Pruessner et al., 2004). Finally, interventions that seek to enhance parental skills may not only reduce psychopathology in children of these families but may also disrupt epigenetic transmission of these behaviors across future generations.

Using randomized control trials David Olds and his colleagues examined the long-term behavioral, emotional, and cognitive consequences of children who were followed by home visiting nurses from the last trimester of their mothers' pregnancies until they were two years old. These children were

compared with a control group that received only prenatal care and routine postnatal developmental screening, but with no intense home nurse visitation. Olds' group examined the long-term effects of this intervention in three separate clinical trials involving three distinct populations: a primarily white sample from semi-rural Elmira, New York (D. Olds et al., 1998), a primarily unmarried low income sample of African American women from Memphis, Tennessee (D. L. Olds, Kitzman et al., 2004), and a more ethnically diverse sample from Denver, Colorado (D. L. Olds, Robinson et al., 2004). Despite some differences in outcomes between the three trials, all show long-term effects of this intervention. The longest follow-up available is provided by the Elmira trial and shows a significant decrease in children's arrest and emerging alcohol use for children assigned to the visiting nurse group as compared to control group at 15-year follow-up (note that this is 13 years after the last nursing visit) (D. Olds et al., 1998). Moreover, both the Memphis and Denver trials show that nurse visitation is associated with a modest improvement in intellectual and language functioning at 6 and 4 year follow-ups respectively [4 and 2 years after the last nurse visit (D. L. Olds, Kitzman et al., 2004; D. L. Olds, Robinson et al., 2004)]. Finally, the Memphis trial also shows significantly fewer behavioral problems in children followed by a nurse at the 6-year follow-up (D. L. Olds, Kitzman et al., 2004). The mechanism by which nurse visitation improves outcome in these children is currently unknown, but as the authors proposed might be mediated by improving several aspects of parental care. These include: improved prenatal health associated with the presence of the nurse during this period, and additional resources for parental investment due to increased length of periods in between additional pregnancies, more stable relationships, and higher rates of employment. Future work may be able to test whether differences between the group receiving nursing visitation and the control group extend also to parent–infant bonds in the following generation, and whether differences in psychopathologies could also be detected in the grandchildren of the parents involved in the initial studies.

Postnatal maternal care affects several behavioral phenotypes: the question of global changes

A fundamental unresolved enigma is how postnatal maternal care is able to influence such a broad range of behavioral phenotypes in the offspring. Work with rodents shows that such sequelae include: fearful response, hippocampal-dependent memory, sexual behavior, maternal care, and vulnerability to substance abuse (see Table 1 and discussion above for details). Similarly, work with non-human primates demonstrates changes in diverse behavioral domains associated with manipulations of maternal care in early life (Barr et al., 2003; Coplan et al., 1995). Finally, extensive clinical data in humans show that child abuse or neglect are associated with increased risk for a large number of psychopathologies such as anxiety, depression, personality disorders, poor impulse control, substance abuse and psychosis (Bebbington et al., 2004; Mullen et al., 1996). The question is then related to the molecular mechanism by which such diverse changes can be induced by maternal care. The answer for this question is currently unknown and unfortunately received very little attention and discussion to date. In some of our earlier publications we suggested that prolonged exposure to high levels of stress hormones mediated by changes in HPA reactivity might be responsible for several of the cognitive and affiliative behaviors associated with maternal care (Meaney, 2001). However, it is also clear that diverse changes in gene expression, such as changes in ER, GABA subunits and NMDA receptors, already exist very early in life and are therefore not likely to be accounted for by a chronic exposure to stress hormones. Alternatively, Saul Schanberg has pointed out that maternal stimulation in the form of LG might be necessary to maintain high levels of several molecules such as growth hormone and ornithine decarboxylase that are necessary to maintain the elevated anabolic state associated with growth of early development (Kuhn & Schanberg, 1998). Our work and work from other labs suggests that low levels of maternal care are associated with low levels of several neurotrophic growth factors such as BDNF and FGF-2 (Bredy, Grant et al., 2003; Liu et al., 2000). These growth factors orchestrate many aspects of development such as cell proliferation, differentiation, migration, survival and rate of synaptogenesis, and are therefore good candidates for molecules able to exert such an extensive change in neurodevelopment. Moreover, some of these neurotrophic factors appear to play important roles in other critical periods such as ocular dominance and tonotopic arrangement of the auditory cortex (Hensch & Fagiolini, 2005; Rubenstein & Merzenich, 2003). Finally, recent work has shown that regulation of BDNF expression in post-mitotic cells is controlled by DNA demethylation of its promoter in an activity-dependent manner (Martinowich et al., 2003). The ability of maternal care to regulate levels of neurotrophic factors provides a simple mechanism to account for its ability to influence such diverse genomic, cellular, and thus behavioral outcomes. Clearly, much work is needed to further test this hypothesis, something that we are currently pursuing in our labs.

Summary

Sensory input during early development plays an important role in brain development with long-term

consequences on brain functioning in adulthood. This environmental form of neuroplasticity appears to be conserved among rodents, non-human primates, and humans, suggesting an underlying similarity in the molecular details that govern this process and the potential of animal models to further understand it. In this context, tactile stimulation provided by the mother early in life appears to play an important role in the development of several behavioral phenotypes that persist into adulthood. Here we discussed two intriguing questions related to these observations. The first question is related to the molecular mechanism by which postnatal maternal care might be able to affect such a diverse array of behavioral phenotypes. The answer to this question is currently unknown and here we propose that the answer might be due to the ability of maternal care to affect expression of neurotrophic factors that in turn control diverse aspects of neurodevelopment. The second question is related to the mechanism by which tactile stimulation early in life influences behavior in adulthood. Recent data has been presented suggesting that the stability of DNA methylation might provide a molecular mechanism in which events early in life program gene expression and therefore behavioral phenotype in a manner that persists into adulthood. The role of ECM in maintaining sequelae of monocular deprivation early in life was also discussed as another mechanism by which sequelae of early life might be maintained across time. Finally, several clinical implications related to these findings have been explored. Of these, we wish to emphasize the need for better understanding of how genes and environment interact to modify neurodevelopment, and how good parenting may improve resiliency to stress.

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