Epigenetics: Behavioral Influences on Gene Function, Part II: Molecular Mechanisms

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This two-part series focuses on the influences of early maternal care on brain development and adult behavior. Specifically, we are interested in behavioral phenotypes established by epigenetic mechanisms (i.e., long-lasting changes in gene function that result from environmental influences). Part I describes the differences in the emotional, maternal, and cognitive behaviors of adult rodent offspring generated by the degree of maternal nurturing that they experience during the first week of life.[1] Animals exposed to a high degree of nurturing show a blunted response to stress, the females are more nurturing mothers, and they have better memories. Part II describes the molecular mechanisms thought to be responsible for these differences in the adult offspring, as well as the molecular mechanisms by which epigenetic effects are propagated from one generation to the next.

MOLECULAR MECHANISMS REGULATE MATERNAL INFLUENCES ON EMOTIONAL BEHAVIOR

Differences arise in the emotional, maternal, and cognitive behaviors of rodents that result from early maternal influences. The influences on emotional behavior appear to be mediated by reactivity in the hypothalamic-pituitary-adrenal axis (HPA). As explained in Part I, the HPA is responsible for changes in the blood levels of corticosterone associated with stress. Environmental stress causes neurons in the hypothalamus to release corticotropin (ACTH)-releasing hormone into the anterior pituitary gland. The anterior pituitary then releases ACTH into the bloodstream, which causes the adrenal cortex to release cortisol, a glucocorticoid. Cortisol mediates the chronic stress response (adrenaline, released from the adrenal medulla, mediates a shorter term stress response). Blood levels of glucocorticoid are a direct measure of HPA reactivity. Several studies reviewed by Kaffman and Meaney[2] have shown that various types of tactile stimulation during the first week of life "blunts" HPA reactivity in adult offspring, whereas the absence of tactile stimulation results in greater HPA reactivity in adult offspring. How does tactile stimulation during the first week of life evoke permanent effects on HPA reactivity and emotional behavior?

GLUCOCORTICOID RECEPTORS (GRs) ARE UP-REGULATED BY TACTILE STIMULATION

GRs are soluble protein molecules present in cells that are sensitive to circulating glucocorticoids. Kaffman and Meaney[2] have shown that the number and density of GRs in nerve cells are modified by tactile stimulation. Specifically, GR expression is up-regulated in the hippocampus and frontal cortex of animals with high levels of tactile stimulation during the first week of life. This is demonstrated by the darker immunocytochemical staining in these structures in animals reared by dams with a high frequency of licking and grooming during the first postnatal week compared with those with less licking and grooming (Fig. 1). Remarkably, the GR up-regulation in these brain regions persists for the animal's lifetime.

The up-regulated GRs in the hippocampus and frontal cortex of rats exposed to high levels of maternal care modify HPA reactivity in these animals by inhibiting (through unknown mechanisms) the input to hypothalamic neurons containing ACTH-releasing hormone. As a consequence, the anterior pituitary does not receive the

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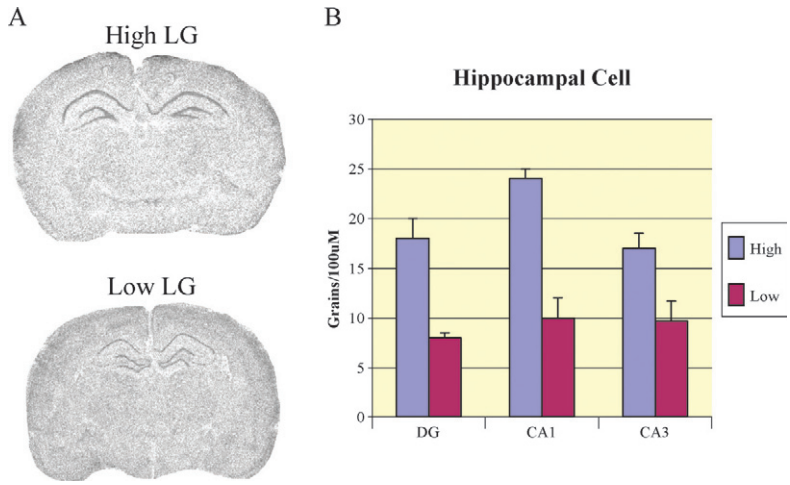

Fig. 1 The maternal behavior of rat dams permanently influences the reactivity of the hypothalamic-pituitary-adrenal axis in her offspring (i.e., a high level of maternal behavior results in less anxious offspring). This effect is mediated by the expression of glucocorticoid receptors (GRs) in the hippocampus. High levels of licking and grooming (LG) and arched-back nursing result in offspring with more GRs in the hippocampus, which blunt hypothalamic-pituitary-adrenal axis reactivity in response to stress, making these animals less anxious. A, High levels of LG result in offspring with more GRs in the hippocampus (top) compared with offspring subjected to low levels of LG (bottom). This pair of photomicrographs depicts high (top) and low (bottom) GR mRNA expression with in situ hybridization methods. B, Quantitative verification of the findings shown in A illustrates that GR mRNA expression is uniformly higher in the offspring of high LG dams in all three regions of the hippocampus tested: DG (dentate gyrus), CA1, and CA3. From Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J Child Psychol Psychiatry*. 2007;48:224–244. Reprinted with permission from Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK and 350 Main Street, Malden, MA 02148.

signal to release ACTH and the adrenal glands secrete less cortisol. Thus, up-regulating GRs "blunts" HPA reactivity. These results can be tested further by examining the brains of pups immediately transferred after birth to the care of either high or low caregivers (cross-fostering). Cross-fostering experiments show that GR expression in brain regions controlling HPA activation is programmed by maternal care, not inborn genetic mechanisms. That is, pups born to dams that provide lower levels of maternal care, but are reared by dams that provide higher levels of care, exhibit blunted HPA activation. What accounts for GR up-regulation in the hippocampus and frontal cortex?

GR up-regulation is a multistep process. The first step occurs during the first week of life in the presence of high levels of tactile stimulation, which increases the circulating levels of the thyroid hormone triiodothyronine. High triiodothyronine levels activate a cyclic adenosine monophosphate–mediated intracellular signaling pathway (involving a serotonin receptor) that ultimately increases the expression of a critical transcription factor known as nerve growth factor–induced clone A (NGFI-A).

Transcription factors are "master control" proteins that regulate the transcription of a number of downstream target genes. Most genes have a regulatory region called a promoter that is responsible for the levels of transcription of that gene. Transcription factors function by binding to specific sequences of nucleotides in the promoter region. Some of these factors repress the expression of genes, whereas others promote transcriptional activity. In this way, a cell can quickly respond to signals that arrive at the cell and change the level of proteins that are required for a specific response.

NGFI-A is a transcription factor that binds to the promoter in the GR gene to activate transcription of these receptors. GRs are rapidly translated from the resulting messages that encode them and are transported to the cell membrane. The increase in NGFI-A expression is transient, occurring only during the first week of life in the presence of high levels of tactile stimulation. Adult animals reared by dams with either high or low levels of maternal care express the same levels of NGFI-A, even though they display long-term differences in HPA reactivity. The explanation for the difference in HPA reactivity in the adult animals exposed to different rearing conditions is that although their NGFI-A levels are the same, the transcription factor has greater access to the GR gene in high licking and grooming/arched-back nursing offspring compared with low high licking and grooming/arched-back nursing offspring. What accounts for this difference in access to the GR gene? DNA methylation.

DNA DEMETHYLATION UP-REGULATES GR EXPRESSION

DNA methylation can be likened to a "molecular fence" that regulates gene expression in all kinds of cells including nerve cells. DNA methylation can interfere with the ability of transcription factors to bind to specific genes. In addition, the methylated DNA recruits a protein complex that further modifies chromatin and leads to a compression and silencing of the underlying genes.

An example of a disorder involving methylation is fragile X syndrome. Fragile X syndrome is one of several disorders characterized by large increases in the number of a particular set of nucleotides. In fragile X syndrome, the increase in nucleotides occurs immediately adjacent to the promoter region of the gene that encodes the fragile X mental retardation protein. The increase is of a particular type: the repeat of a triplet of nucleotides, which in the case of fragile X are the three nucleotides C-G-G. It turns out that CpG sequences are the target of methylation because the added methyl group occurs only to cytosine residues. The net effect is a dramatic increase in DNA methylation. Because methylation leads to a tight compression and inactivation of this stretch of DNA, the result is that no message is transcribed and no protein is translated. The result is the lack of the fragile X syndrome protein, which is critically involved in synaptic plasticity (exactly how this protein functions is discussed in the fourth column in this series).

These chemical modifications can persist for extended periods. A central point is that these modifications have a profound effect on gene expression. Researchers examining the effects of maternal care on adult behavior have shown that shortly after birth, the region of DNA encoding the GR in the forebrain and hippocampus is highly methylated. The methylation occurs in the region that normally would allow the binding of the transcription factor NGFI-A. Methylation, however, makes the gene inaccessible and limits the expression of GRs. Exposure to high maternal care during the first postnatal week actually reduces the level of DNA methylation in this region (Fig. 2). This state of DNA demethylation is long-lasting, persisting throughout the lifetime of the animal. DNA methylation decreases GR expression, which results in increased HPA reactivity. DNA demethylation allows NGFI-A to bind to the GR promoter, which increases GR expression and decreases HPA reactivity. Remember that up-regulated GRs in the hippocampus and frontal cortex modify HPA reactivity by inhibiting the input to hypothalamic neurons containing ACTH-releasing hormone, thereby blunting HPA reactivity. The key here is that the amount of NGFI-A is the same in both groups; it is the ability of NGFI-A to bind to the DNA that differs. In short,

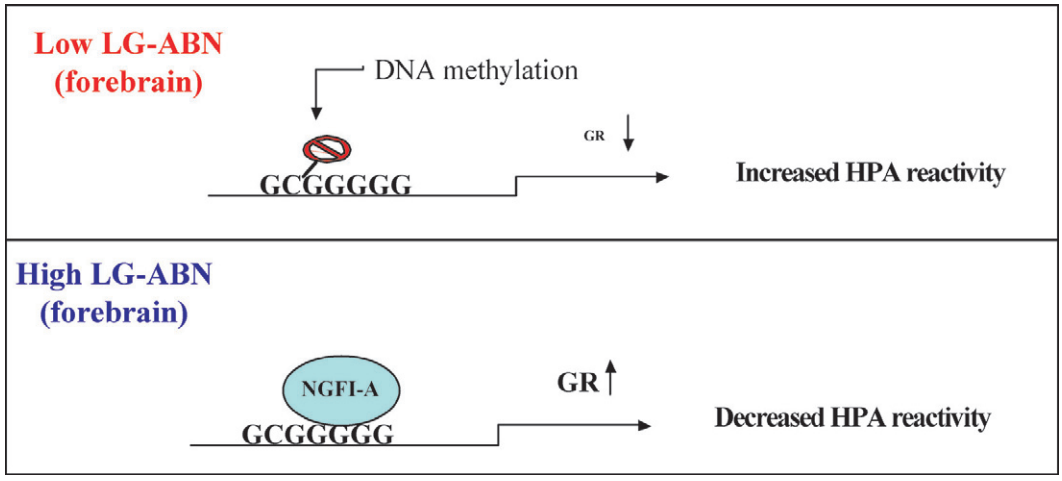

Fig. 2 DNA methylation and demethylation regulate the expression of glucocorticoid receptor (GR) expression and hypothalamic-pituitary-adrenal axis (HPA) reactivity in rats. Low levels of licking and grooming and arched-back nursing (LG-ABN) methylate the GR promoter (top), preventing GR expression in the hippocampus. In contrast, high levels of LG-ABN result in demethylation of the GR promoter (bottom), activating GR expression in the hippocampus. An increase in GR in the hippocampus blunts HPA reactivity. This DNA methylation pattern is established in the first week of life in response to maternal behavior and is permanent. NGFI-A = nerve growth factor–induced clone A. From Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J Child Psychol Psychiatry*. 2007;48:224–244. Reprinted with permission from Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK and 350 Main Street, Malden, MA 02148.

changes in DNA methylation have stable, long-lasting effects on GR gene expression. Because of its molecular stability, the effect of DNA methylation on GR expression is a perfect epigenetic mechanism for altering vulnerability to stress for long periods of time, if not permanently.

MOLECULAR MECHANISMS REGULATE TRANSGENERATIONAL INFLUENCES ON MATERNAL BEHAVIOR

As noted in Part I, female offspring reared by nurturing mothers become nurturing dams themselves. This maternal phenotype is as stable as the change in HPA reactivity described above and is also the result of changes in DNA methylation. Cross-fostering experiments show that it is the foster mother, not the biological mother, who confers this phenotype on her young. This effect is mediated by an estrogen receptor (called ER-α) in a region of the hypothalamus of the female rat known as the medial preoptic area (MPOA). A portion of the ER-α gene, called the promoter, is methylated or demethylated depending on whether the mother was poor or excellent at maternal care (Fig. 3). This figure shows that in the presence of the elevated estrogen associated with pregnancy, female offspring of high-nurturing mothers have higher levels of activated ER-α in the MPOA. ER-α activation has important downstream effects on oxytocin receptor binding in the MPOA, which mediates labor, lactation, and maternal behavior. When the ER-α DNA is demethylated (in the offspring of nurturing mothers), higher levels of postpartum oxytocin receptors in the MPOA result in higher levels of maternal behavior. In other words, activation of oxytocin receptors in the MPOA, secondary to up-regulation of ER-α, is responsible for individual differences in maternal behaviors. It is by this mechanism that the effects of maternal behavior become transgenerational.

MOLECULAR MECHANISMS REGULATE MATERNAL INFLUENCES ON COGNITIVE BEHAVIOR

Adult offspring of nurturing dams have better memories than offspring of low-nurturing dams. Changes in the hippocampus, a brain region involved in learning and memory, are responsible for these differences. There are multiple subtypes of glutamate receptors, but the two glutamate receptors relevant to this discussion are the *N*-methyl-d-aspartate (NMDA) and amino-3-hydroxy-5-methyl-d-aspartate (AMPA) receptors. The electrical activity of hippocampal neurons is modified by the activity and the number of these two receptor types. NMDA receptors become activated only under certain conditions, such as a strong or high-frequency stimulus. Activation of these NMDA receptors favors long-term electrophysiological changes in the hippocampal neurons (called long-term potentiation) that are necessary for

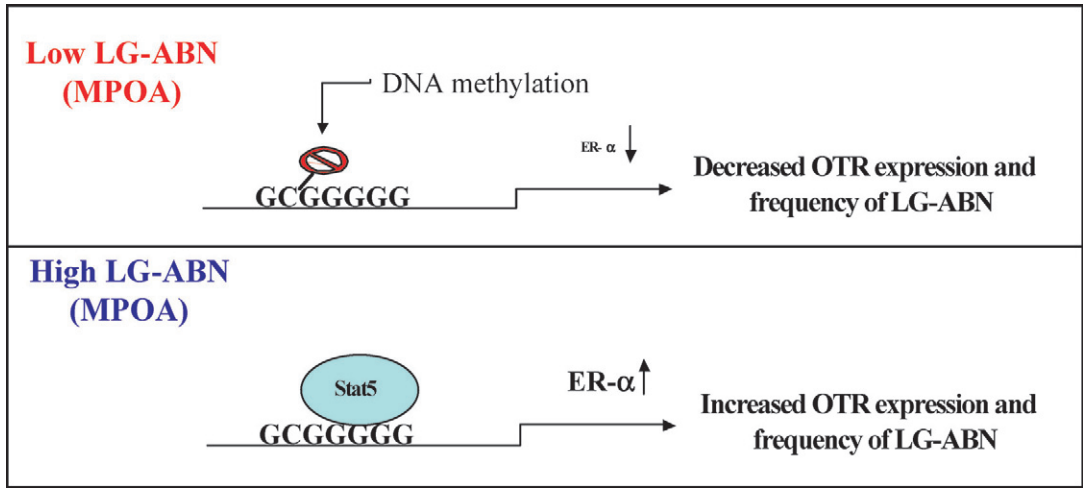

Fig. 3 Female offspring of high versus low licking and grooming and arched-back nursing (LG-ABN) dams develop stable maternal phenotypes that correspond to their experience as rat pups. High levels of LG-ABN (bottom) results in demethylation in the estrogen receptor α (ER-α) promoter in the medial preoptic area (MPOA) of the hypothalamus. The downstream effect of demethylation is increased expression of oxytocin receptors in the MPOA and increased LG-AGN behaviors in these female offspring when they produce a litter. The opposite occurs in females raised by low LG-ABN dams (top). In this way, maternal care early in life establishes a maternal phenotype in female offspring and epigenetic transmission of this phenotype across generations. From Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J Child Psychol Psychiatry*. 2007;48:224–244. Reprinted with permission from Blackwell Publishing, 9600 Garsington Road, Oxford OX4 2DQ, UK and 350 Main Street, Malden, MA 02148.

learning to take place. When NMDA receptors are activated in this way, the number of AMPA receptors increases. The increase in AMPA receptors enhances the efficacy of the neuronal connections (making an action potential more likely), facilitating memory. As a consequence, a situation that previously required a large or high-frequency stimulus now activates the neurons in the presence of less powerful stimuli.

Several studies have shown[2] that maternal care by nurturing dams improves learning in the offspring by enhancing NMDA-mediated activity in the hippocampus. Offspring of low-nurturing dams demonstrate poorer performance because they do not undergo the same changes in NMDA receptors. Remarkably, this effect can be reversed by exposing the animals to an enriched environment during the prepubescent periods. In the right environment, animals deprived of high-quality maternal care can become indistinguishable from the offspring that received it; but exposing offspring of nurturing dams to an enriched environment does not change their performance on hippocampal cognitive tasks. Their superior behavior remains superior but no better. Researchers have shown that the improvement in learning is the result of a compensatory increase in AMPA receptor number, not changes in the NMDA receptors.[2] Therefore, the mechanisms mediating the initial effect and the compensatory effect are different. Whether these changes are associated with DNA methylation is not known.

IMPLICATIONS FOR HUMAN BEHAVIOR

Kaffman and Meaney have suggested that the effects described here are conserved among rodents, nonhuman primates, and humans.[2] DNA methylation appears to program gene expression in response to environmental factors early in life and to promote behavioral phenotypes that persist into adulthood. The implication of these events cannot be overemphasized, as we have long wondered how it was that environmental influences affect behavior. The question addressed here is how parenting affects the stress response and other behavior and, ultimately, how these effects can be modified for better clinical outcomes. The larger issue is that epigenetic effects on gene expression clearly influence brain development in ways that correspond to the early environment. The molecular mechanism by which nurture influences nature is finally being uncovered.

Disclosure: The authors report no conflicts of interest.

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