2 Interplay Between Social Experiences and the Genome: Epigenetic Consequences for Behavior

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ABSTRACT

Social experiences can have a persistent effect on biological processes leading to phenotypic diversity. Variation in gene regulation has emerged as a mechanism through which the interplay between DNA and environments leads to the biological encoding of these experiences. Epigenetic modifications—molecular pathways through which transcription is altered without altering the underlying DNA sequence—play a critical role in the normal process of development and are being increasingly explored as a mechanism linking environmental experiences

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to long-term biobehavioral outcomes. In this review, evidence implicating epigenetic factors, such as DNA methylation and histone modifications, in the link between social experiences occurring during the postnatal period and in adulthood and altered neuroendocrine and behavioral outcomes will be highlighted. In addition, the role of epigenetic mechanisms in shaping variation in social behavior and the implications of epigenetics for our understanding of the transmission of traits across generations will be discussed.

Though our DNA sequence provides a template for generating individual differences and for the transmission of those characteristics across generations, it is evident that the environment contributes significantly to both of these processes. The dichotomy between the influences of nature (DNA) and nurture (environment) has become part of our theoretical framework for understanding the origins of our unique characteristics—ranging from personality to disease risk. However, advances in molecular biology have provided enlightenment regarding both the relationship between genotype and phenotype and the mechanisms through which environment shapes biological processes, and the insights gained from these advances has challenged the nature versus nurture dichotomy. It is becoming increasingly apparent that development is a process of dynamic interplay between the genome and our environmental experiences. This interplay is perhaps best illustrated in the concept of epigenetics—the study of those factors which alter the activity of genes without altering the underlying DNA sequence. There is increasing evidence that environmental experiences can come to shape the activity of genes through epigenetic pathways and thus the genotype to phenotype and environment to phenotype relationships may ultimately be governed by similar molecular processes.

The investigation of environmentally induced epigenetic effects has highlighted the impact of a wide variety of environmental exposures, including nutritional levels during development, toxins, and both early and later life stressors (Champagne, 2010; Jirtle and Skinner, 2007). However, it is perhaps the interplay between social experiences and the genome which demonstrates most profoundly how nature and nurture interact. Across a variety of species, there is evidence for the effect of social experiences occurring across the lifespan on epigenetic pathways leading to broad phenotypic effects, including stress responsivity, learning/memory, and reproductive behavior (Champagne, 2010). Moreover, epigenetic mechanisms may account for the emergence of social experiences through the effects of these molecular modifications on social behavior (Auger *et al.*, 2011; Kataoka *et al.*, 2011). Ultimately, these interactive effects can lead to the transgenerational continuity of individual differences in neurobiological and behavioral characteristics (Champagne, 2008). In this chapter, evidence for the epigenetic consequence of social experiences, such as mother–infant interactions and adult social interactions, will be highlighted followed by a discussion of the epigenetic basis of variation in social behavior.

This research contributes to our understanding of the inheritance of behavior and raises challenging questions regarding future directions in the study of behavioral epigenetics.

I. EPIGENETIC MECHANISMS AND DEVELOPMENT

The regulation of gene transcription is a dynamic process and illustrates the complexity of the genotype to phenotype relationship. Epigenetic modifications are a key component of this process and can act to enhance or suppress transcription through a variety of pathways. The term "epigenetic" has historically been used to describe the interplay between genes and gene products which can result in variation in the phenotype of an organism (Jablonka and Lamb, 2002; Waddington, 1942). The term predates modern approaches to genetics and suggests that there are factors "over" or "upon" genetic variation that must be considered within the study of developmental biology. However, more modern uses of the term are in the description of the specific molecular mechanisms that, through interactions with DNA or DNA products (mRNA), lead to variation in gene expression. Though our understanding of the complexity and variety of these mechanisms is rapidly expanding, here the focus will be on epigenetic factors that have been studied in the context of developmental plasticity in behavior in response to the environment and variation in social behavior: DNA methylation, histone modifications, and microRNAs (miRNAs).

A. DNA methylation

The accessibility of DNA to transcription factors and RNA polymerase is a critical step in the initiation of transcription. DNA methylation is a molecular modification which alters this accessibility without altering the underlying DNA sequence (Turner, 2001). Cytosine nucleotides within the DNA sequence can become methylated through the chemical addition of a methyl group to the 5 position of the cytosine pyrimidine ring (see Fig. 2.1A). This modification is not a mutation—the cytosine can still make an appropriate base pairing with guanine—however, the cytosine becomes less accessible to transcription factors. Moreover, methylated DNA attracts methyl-binding proteins (MBDs), such as methyl-CpG-binding protein 2 (MeCP2), which can further modify the chromatin structure to reduce transcription (Fan and Hutnick, 2005; Razin, 1998). DNA methylation typically occurs at CpG dinucleotides, which are often found in gene promoters (i.e., DNA sequences upstream from the transcription start site which regulate gene activity) (Deaton and Bird, 2011). The addition of methyl groups to cytosines is accomplished through the actions of the DNA methyltransferases (DNMTs). Within this class of enzymes are the maintenance

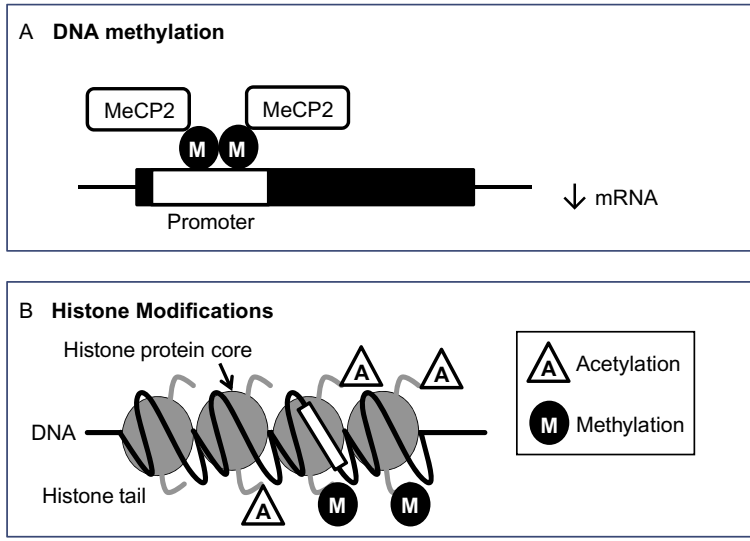

Figure 2.1. Schematic illustration of DNA methylation and histone modifications. (A) DNA methylation involves the formation of a chemical bond between a methyl group (M) and cytosines within the DNA sequence. Methylated cytosines attract methyl-binding proteins such as MeCP2. When cytosine methylation occurs within the promoter region of a gene, there is typically a reduction in transcriptional activity of the gene leading to reduced mRNA levels. (B) DNA is wrapped around a core of histone proteins. The amino acid tails of these proteins can undergo multiple posttranslational modifications, including acetylation (A) and methylation (M). These modifications may alter the interactions between histone tails and the surrounding DNA sequence resulting in altered gene transcription.

DNMTs—such as DNMT1—which methylate hemimethylated DNA following DNA replication as well as the *de novo* methyltransferases (DNMT3a/b) which add methyl groups to unmethylated DNA (Okano *et al.*, 1999; Turek-Plewa and Jagodzinski, 2005). The mechanism of removal of methyl marks within DNA has been far more elusive, particularly in light of the strong covalent bond which characterizes this chemical modification. However, during DNA replication and repair, there are opportunities for the passive loss of DNA methylation. Drugs that inhibit DNMT1—such as 5-azacytidine—have been demonstrated to reduce DNA methylation levels, oxidation of 5-methylcytosine to 5-hydroxymethylcytosine may promote subsequent demethylation, and recent evidence suggests there may be specific enzymes with demethylase properties which can induce DNA demethylation in postmitotic cells (Nabel and Kohli, 2011; Wu and Zhang, 2010). Thus, there is the capacity to both add and remove methyl groups to/from DNA with implications for transcriptional activity.

DNA methylation is a fundamental process within development and functions to maintain patterns of cellular differentiation. This function is accomplished via two important properties of DNA methylation—stability and heritability. Though there are processes which can lead to demethylation and genome-wide demethylation is observed in most species during early embryonic development (Santos *et al.*, 2002), DNA methylation is considered a mechanism of stable gene silencing (Razin, 1998). Moreover, DNA methylation patterns can be maintained following mitosis, thus permitting stability of cell type-specific gene expression profiles (Jones and Taylor, 1980). During the rapid phases of cell proliferation and differentiation that occur during early embryonic development, the process of DNA methylation is essential for survival. Targeted deletion of *DNMT1* or *DNMT3a/b* in mice has been found to be lethal and results in abnormal gene expression patterns (Li *et al.*, 1992; Okano *et al.*, 1999).

B. Histone modifications

The compact storage of DNA within the cell nucleus is accomplished through the condensation of chromatin via the tight packaging of nucleosomes, which are the fundamental units of chromatin and consist of DNA wrapped around a protein core. The protein core of the nucleosome is made up of the histone proteins H2a, H2b, H3, and H4 (Turner, 2001). The N-terminal "tails" of histone proteins can undergo multiple posttranslational modifications which can alter chromatin structure (see Fig. 2.1B). These modifications typically occur at lysine residues throughout the tail and include acetylation, methylation, ubiquitination, and phosphorylation (Jenuwein and Allis, 2001; Peterson and Laniel, 2004). The nature and the location of the modification will determine the effect of this epigenetic modification for gene transcription. For example, acetylation of lysines within the H3 histone is typically associated with increased transcriptional activity, whereas H3 methylation can reduce transcriptional activity (e.g., H3K9 methylation) or be associated with active gene expression (e.g., H3K4 methylation; Barski *et al.*, 2007; Koch *et al.* 2007). The availability of enzymes to facilitate these posttranslational modifications is a key regulatory step in histone-mediated effects (Legube and Trouche, 2003). In the case of acetylation, the histone acetylases promote the occurrence of this modification, whereas histone deacetylases (HDACs) remove the acetyl groups from the histone tails. Similarly, there are enzymes that promote histone methylation (i.e., histone methyltransferases), and enzymes that remove the methyl groups from histone proteins (i.e., histone demethylases). There are multiple types of HDACs (e.g., HDAC1, HDAC5) and histone methyltransferases/demethylases (e.g., EHMT1, JARID1C), defined by tissue and substrate specificity, and mutation of the genes encoding these enzymes can have a profound effect on development (Dokmanovic *et al.*, 2007). Pharmacological manipulation of histone acetylation

can be achieved through use of valproic acid, sodium butyrate, and trichostatin A—compounds that inhibit HDACs (Monneret, 2005). The modification of histones is dynamic and can lead to transient and reversible effects on transcription.

C. MicroRNA

miRNAs are small, noncoding RNA molecules which can interfere with mRNA molecules and thus act to modify gene activity through posttranscriptional modification. miRNAs can suppress gene activity by binding to the mRNA of multiple target genes and preventing translation, cleaving the mRNA, or promoting mRNA degradation (Sato *et al.*, 2011). An increasing number of miRNAs have been identified (e.g., miR-9, miR-137) which have specificity with regard to the range of target genes that are repressed by these molecules. Though perhaps not classically "epigenetic" in the sense that miRNAs typically target the product of transcription rather than interacting directly with the DNA, miRNAs play a significant role in gene regulatory networks and thus are an important consideration in the pathways linking genotype to phenotype.

D. Interaction between epigenetic pathways

Though a typical approach to the study of epigenetic mechanisms is to consider each separately, these factors are acting in concert to regulate gene expression and there are dynamic interactions between DNA methylation, posttranslational histone modifications, and miRNAs. For example, MBDs attract a protein complex to methylated DNA which includes HDACs (Fan and Hutnick, 2005; Razin, 1998). Consequently, DNA methylation often coincides with histone deacetylation. Pharmacological inhibition of HDACs has also been demonstrated to reduce levels of DNA methylation (Alonso-Aperte *et al.*, 1999; Selker, 1998). miRNAs are known to be regulated by epigenetic modifications and to exert epigenetic modifications on the expression of HDACs and DNMTs (Sato *et al.*, 2011). These interactions, combined with the complexity of each individual modification, make the prediction of gene expression based on any single epigenetic modification a challenging undertaking.

II. EPIGENETIC IMPACT OF SOCIAL EXPERIENCES

Though epigenetic mechanisms can confer a high degree of both plasticity and stability to gene expression patterns, the flexibility of these mechanisms (particularly DNA methylation) in response to environmental cues was initially thought to be limited to disruptions occurring during early embryonic

development. However, it has been well established that stable individual differences in gene expression can be observed as a consequence of environmental experiences occurring across the lifespan (Covington *et al.*, 2009; Lippmann *et al.*, 2007; Meaney, 2001; Seckl and Meaney, 2004). Moreover, there is increasing support for the hypothesis that epigenetic mechanisms may be altered by a wide range of environmental events and thus mediate the long-term effects of the environment on biological and behavioral outcomes (Champagne, 2010; Jirtle and Skinner, 2007; Meaney and Szyf, 2005). In humans, the study of monozygotic twins, which has been a classic methodological tool within genetics, has provided intriguing insights into the potential for epigenetic plasticity in driving phenotypic divergence. DNA methylation and histone acetylation patterns are highly concordant among young twins (<28 years of age) but diverge significantly among older twins (>28 years of age), leading to the speculation that this divergence emerges across the lifespan in response to the unique environmental experiences of each twin (Fraga *et al.*, 2005).

Though there are multiple features of the environment that can shape development, an intriguing question has been regarding the mechanisms through which our social experience come to be embedded in our biology. Decades of longitudinal and laboratory-based studies have highlighted the association between the quality of the social environment and neurobiological and behavioral outcomes (Ammerman *et al.*, 1986; Miller *et al.*, 2009; Pruessner *et al.*, 2004; Sroufe, 2005; Trickett and McBride-Chang, 1995). The mediating role of epigenetic mechanisms in this association is becoming increasingly apparent, based primarily on evidence from animal models in which the quality of the social environment can be manipulated and brain region-specific epigenetic modifications assessed. In particular, these studies indicate epigenetic effects induced by the quality of postnatal mother–infant interactions and the experience of agonistic social encounters in adulthood.

A. Epigenetic effects of mother–infant interactions

The quality of social interactions occurring early in development can have a profound developmental effect. In humans, this effect is demonstrated by the severe cognitive and social deficits that emerge among infants that experience childhood neglect or abuse (Eluvathingal *et al.*, 2006; MacLean, 2003; Trickett and McBride-Chang, 1995). Disruption to the parent–infant relationship can lead to impairments in attachment—the formation of a relationship with a primary caregiver which promotes social/emotional development—with subsequent effects on the risk of psychopathology (Bowlby, 1988; Egeland and Farber, 1984; Johnson *et al.*, 2000). Parental sensitivity to infant cues is a key element within the formation of an attachment relationship, and variation in maternal sensitivity within the normal range has been found to predict stress sensitivity

and negative affect among infants (Hane and Fox, 2006; Pederson *et al.*, 1998). Through the use of neuroimaging techniques, the neurobiological consequences of disruption or variation in the mother–infant relationship are becoming increasingly apparent (Chugani *et al.*, 2001; Eluvathingal *et al.*, 2006; Pruessner *et al.*, 2004). However, empirical studies of the molecular and epigenetic consequences of variation in the early social environment have been based primarily on rodent models of these experiences.

1. Variation in maternal care

Among most species, there are significant variations in the quality and frequency of parent–offspring interactions during the postnatal period (Champagne *et al.*, 2003a, 2007; Fairbanks, 1989; Hane and Fox, 2006; Maestripieri, 1998). These individual differences in parental care can be influenced by environmental factors, such as stress, food availability, and the social context of the rearing environment (Champagne and Meaney, 2006, 2007; Curley *et al.*, 2009; Gorman *et al.*, 2002). Though there are species that engage in biparental or exclusive paternal care of offspring, for most species, the rearing of offspring is accomplished exclusively through mother–infant interactions. Among Long-Evans laboratory rats, the long-term and epigenetic consequences of maternal care have been explored and provide empirical support for the hypothesis that the quality of mother–infant interactions can lead to changes in DNA methylation and histone acetylation (Meaney and Szyf, 2005; Weaver *et al.*, 2004). During the postnatal period, maternal care in rats is characterized by frequent nursing and licking/grooming (LG) of offspring. LG provides a source of tactile stimulation for offspring that alters physiology and promotes urination (Gubernick and Alberts, 1983; Sullivan *et al.*, 1988a,b). Though a minimal level of LG is needed to achieve these physiological roles, it is evident that even within the stable conditions of laboratory housing, there are individual differences in the frequency with which lactating female rats will engage in this behavior (Champagne *et al.*, 2003a). This variation can be used to study the impact of experiencing low levels of LG (Low LG) versus high levels of LG (High LG) on multiple neurobiological and behavioral outcomes in offspring (see Fig. 2.2). Male offspring of Low LG dams are observed to have a heightened hypothalamic–pituitary–adrenal (HPA) response to stress, impaired cognition, and reductions in hippocampal plasticity in adulthood (Caldji *et al.*, 2000; Champagne *et al.*, 2008; Liu *et al.*, 1997, 2000). Female offspring reared by Low LG dams have decreased estrogen sensitivity in the medial preoptic area of the hypothalamus (MPOA) in adulthood and display reduced levels of care toward their own offspring (Champagne *et al.*, 2001, 2003a,b). Moreover, cross-fostering studies indicate that these long-term outcomes are associated with the quality of maternal care experienced during the postnatal period rather than genetic or prenatal factors.

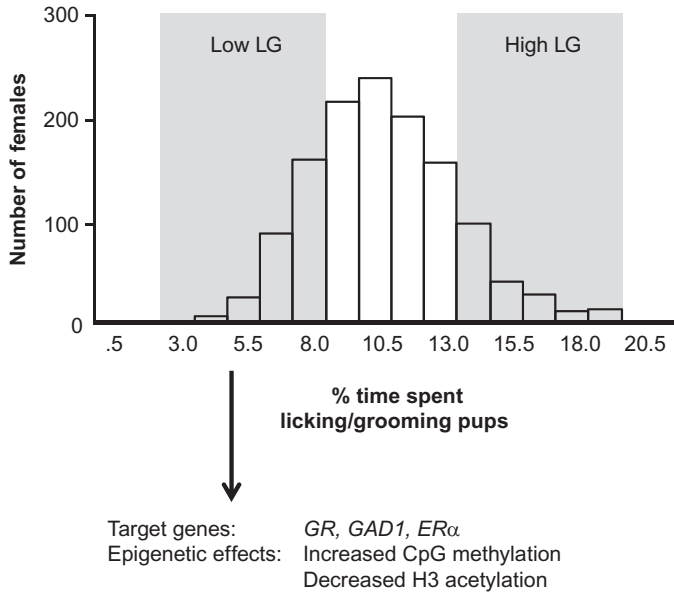

Figure 2.2. Summary of the epigenetic effects of natural variations in licking/grooming (LG) experienced during postnatal development. Lactating female rats engage in variation in the frequency of LG toward pups. Offspring reared by Low LG compared to High LG females can be compared on molecular, neurobiological, and behavioral measures. The experience of Low LG during infancy is associated with increased CpG methylation and decreased histone acetylation within the glucocorticoid receptor (*GR*) and glutamic acid decarboxylase (*GAD1*) gene promoter regions in the hippocampus of male offspring and the estrogen receptor alpha (*ERα*) promoter in the hypothalamus of female offspring.

To elucidate the potential role of epigenetic mechanisms in these maternal effects, gene regulation in the hippocampus of male offspring and MPOA of female offspring has been explored. The heightened HPA response to stress characteristic of offspring reared by Low LG dams is associated with impairments in HPA negative feedback (Liu *et al.*, 1997). Within the hippocampus, glucocorticoid receptor (GR) levels are a key regulator of HPA negative feedback with a higher density of GR associated with enhanced ability to down-regulate the stress response (Sapolsky *et al.*, 1985). Among adult male offspring of Low LG dams, there are reduced mRNA and protein levels of hippocampal GR which may account for the heightened stress responsivity observed in these offspring (Francis *et al.*, 1999). Analysis of the *GR* 1_7 promoter region suggests that LG may exert these long-term effects on *GR* expression through DNA methylation (Weaver *et al.*, 2004). Offspring that receive low levels of maternal

LG during the postnatal period have increased hippocampal *GR* 1_7 promoter DNA methylation at several CpG sites in this region. In particular, the binding site for the transcription factor NGFI-A (nerve growth factor-inducible protein A, also known as EGR-1 and ZIF268) is highly methylated among the offspring of Low LG dams and almost completely unmethylated among the offspring of High LG dams. These adult patterns of DNA methylation are not observed prenatally or at the time of birth but instead emerge during the first postnatal week, during which time the differential maternal LG behavior is most pronounced. Histone H3 acetylation at lysine 9 (H3K9) is also reduced within the *GR* 1_7 promoter of Low LG offspring as is the binding of NGFI-A to this region. These epigenetic effects can be reversed through ICV administration of the HDAC inhibitor trichostatin A into the hippocampal region of adult Low LG offspring (Weaver *et al.*, 2004). Similarly, the ICV administration of methionine—a methyl donor—can increase *GR* 1_7 DNA methylation, decrease NGFI-A binding to the *GR* 1_7 promoter region, and decrease *GR* mRNA and protein levels in the adult offspring of High LG dams (Weaver *et al.*, 2005)—illustrating the potential plasticity of these epigenetic effects.

Within the hippocampus of adult male offspring of Low LG dams, there are also elevated levels of *DNMT1* mRNA (Zhang *et al.*, 2010), providing a possible mechanism for global changes in DNA methylation and gene expression. Indeed, genome-wide expression assays indicate that over 900 genes are differentially expressed in the hippocampus as a function of maternal LG, and that overall there is increased gene expression among offspring of High LG compared to Low LG dams (Weaver *et al.*, 2006). In addition to hippocampal *GR*, maternal LG has been found to alter expression of the enzyme glutamic acid decarboxylase (*GAD1*) gene, which may account for the maternal effects on hippocampal γ-aminobutyric acid circuits and receptor subunit composition (Caldji *et al.*, 2003; Zhang *et al.*, 2010). Analysis of DNA methylation within CpG sites of the *GAD1* promoter region indicates that offspring reared by Low LG dams have heighted DNA methylation and reduced H3K9 acetylation in this region (Zhang *et al.*, 2010). Thus, variation in maternal care can have widespread effects on gene expression which likely involve maternal effects on epigenetic modifications within the promoter regions of differentially expressed genes.

Maternal LG effects on female offspring have primarily focused on reproductive outcomes such as sexual and maternal behavior (Cameron *et al.*, 2008a,b; Champagne *et al.*, 2003a). Among female offspring of Low LG dams, there are decreased mRNA and protein levels of estrogen receptor alpha (ERα) in the MPOA, which may account for the reduced estrogen sensitivity and maternal care evident in these offspring (Champagne *et al.*, 2001, 2003b). Analysis of levels of DNA methylation within the 1B promoter region of the *ERα* gene in MPOA tissue indicates that offspring of Low LG dams have elevated CpG methylation within this region (Champagne *et al.*, 2006). As was the case for *GR*, these rearing

environment-associated changes in DNA methylation alter the capacity for transcription factors to gain access to gene promoter regions. Among offspring of Low LG dams, there is decreased binding of the transcription factor STAT5a (signal transducer and activator of transcription 5A) to the *ERα* 1B promoter (Champagne *et al.*, 2006). Thus, the differential DNA methylation associated with maternal LG likely has functional consequences for transcription factor-mediated upregulation of gene expression in the MPOA.

2. Maternal separation

The classic studies of Harlow demonstrated the dramatic effect of early life maternal deprivation on development in rhesus macaques (Harlow *et al.*, 1965; Seay and Harlow, 1965), effects that are consistent with observations of the effects of social neglect on development in human infants. Epigenetic effects of maternal deprivation (through rearing of infant rhesus macaques in a nursery) have also been demonstrated. It has been noted in humans and rhesus that genetic polymorphisms in the serotonin transporter (*5-HTT*) gene promoter can lead to differential susceptibility to the effects of stress such that carriers of the short allele (which is predictive of less expression of *5-HTT*) show heightened stress responsivity and increased risk of depression when exposed to increasing life stress, childhood maltreatment, or, in the case of rhesus, maternal deprivation (Aguilera *et al.*, 2009; Caspi *et al.*, 2003). DNA methylation levels within CpGs of the *5-HTT* promoter in peripheral blood mononuclear cells collected from 90- to 120-day-old macaques were found to be higher in carriers of the short version of the *5-HTT* allele (regardless of early rearing condition), and *5-HTT* CpG methylation was found to interact with rearing condition to predict behavior. Elevated DNA methylation combined with maternal deprivation was found to predict heightened stress reactivity (Kinnally *et al.*, 2010). Though it is yet unclear how to interpret the biological meaning of peripheral tissue measures of DNA methylation, these findings do suggest a potential role for epigenetic programming in the long-term effects of early life deficits in the social environment.

Maternal separation studies in mice have provided an opportunity to determine the brain region-specific epigenetic alterations that are induced through the experience of reductions in maternal care. In rodents, prolonged maternal separation (1–3 h per day) throughout the postnatal period has been found to induce changes in multiple neuroendocrine and neuropeptide systems and lead to heighted HPA response to stress (Lehmann and Feldon, 2000; Lippmann *et al.*, 2007). Within the parvocellular neurons of the paraventricular hypothalamus (PVN), maternal separation has been found to induce persistent increases in arginine vasopressin (*AVP*) mRNA (Murgatroyd *et al.*, 2009). Within the *AVP* gene, there are four regions rich in CpG islands that could

potentially regulate gene expression through DNA methylation. Among male offspring that experienced maternal separation, DNA methylation is reduced at one of these four regions (CGI3) at 6 weeks, 3 months, and 1 year of age (Murgatroyd *et al.*, 2009). This maternal effect may be PVN specific as rearing environment was not found to induce changes in *AVP* mRNA or DNA methylation in the supraoptic nucleus. Subsequent analyses indicated that the activation of MeCP2 (through phosphorylation of this MBD) may be a critical factor within these pathways, leading to *AVP* hypomethylation and increased *AVP* mRNA levels within the PVN. In a similar maternal separation paradigm in mice, male offspring exposed to prolonged separation from dams and reduced maternal care consequent to this manipulation were found to have increased DNA methylation within several CpG sites of the *MeCP2* and cannabinoid receptor-1 (*CB-1*) genes and decreased CpG methylation within the corticotropin-releasing factor receptor-2 (*CRFR2*) gene (Franklin *et al.*, 2010). These epigenetic effects were detected in sperm cells of maternally separated males. Interestingly, within the cortex of the female offspring of maternally separated males, increased DNA methylation in *MeCP2* and *CB-1* genes and decreased DNA methylation of *CRFR2* were detected, possibly indicating the inheritance of epigenetic modifications by these offspring via the germ cells of maternally separated males.

3. Maternal abuse

The long-term consequences of childhood abuse that have been documented in humans and in primates illustrate the impact of agonistic social encounters occurring in infancy (Ammerman *et al.*, 1986; Maestripieri, 2005). Laboratory rodent models of abuse have typically manipulated the quality of the rearing environment by restricting the amount of nesting materials available to dams. This disruption to the postnatal environment induces reductions in maternal care and an increased incidence of dams stepping on pups, aggressive grooming, and dragging of pups by a limb (Brunson *et al.*, 2005; Ivy *et al.*, 2008; Raineki *et al.*, 2010). Similar to maternal separation, exposure to this rearing environment induces heightened stress responsivity and impairments in spatial memory (Avishai-Eliner *et al.*, 2001; Brunson *et al.*, 2005; Gilles *et al.*, 1996; Raineki *et al.*, 2010). Using a variation of this methodology with rats in which offspring are exposed to a brief encounter with an abusive nonbiological mother, the epigenetic effects of maternal abuse have been demonstrated. Male and female offspring that experience increased abusive behaviors in infancy have decreased brain derived neurotrophic factor (*BDNF*) mRNA levels within the prefrontal cortex in adulthood and within the IV promoter region of the *BDNF* gene, and abuse is associated with increased CpG methylation (Roth *et al.*, 2009). Females that experience abusive rearing conditions are themselves more likely to engage in abusive behavior, and

the offspring of abused females likewise have increased *BDNF* IV promoter DNA methylation in the prefrontal cortex, suggesting that environmentally induced epigenetic changes can be transmitted across generations.

4. Enhancing mother–infant interactions

Models of early life adversity are certainly most prevalent in the animal literature, yet there are also paradigms which lead to increased nurturing mother–infant interactions. Though prolonged maternal separation from offspring leads to reduced maternal care and heightened HPA response to stress in offspring, brief separations may stimulate maternal care, particularly LG, and attenuate offspring stress responsivity (Lehmann *et al.*, 2002; Meaney *et al.*, 1991). In rodents, brief maternal separation (also called *handling*) results in reductions in corticotropin-releasing factor (*CRF*) mRNA in the parvocellular neurons of the PVN that can be observed as earlier as postnatal day 9 (PN9) (Korosi *et al.*, 2010). Within the regulatory region of the rat *CRF* gene resides a binding element (NRSE) for the repressor neuron-restrictive silencer factor (NRSF) (Seth and Majzoub, 2001) and studies in human cell lines have shown that when NRSF is bound to this region, there is recruitment of cofactors and other enzymes/proteins involved in epigenetic regulation leading to the repression of gene expression (Zheng *et al.*, 2009). Among handled offspring, protein levels of NRSF are dramatically higher in PVN tissue at PN9 and throughout adulthood, suggesting a possible mechanism for handling induced reductions in *CRF* gene expression (Korosi *et al.*, 2010). Maternal care can likewise be stimulated through communal rearing, in which multiple lactating females rear offspring in a communal nest. In mice, this paradigm has been demonstrated to increase the frequency with which pups receive LG and nursing and leads to long-term changes in neurobiology and behavioral outcomes (Branchi, 2009; Curley *et al.*, 2009). Offspring that have been reared in a communal nest have increased hippocampal histone H3 acetylation at the *BDNF* I, IV, VI, and VII promoter regions, and this epigenetic modification may account for the increased *BDNF* levels observed among communally reared mice (Branchi *et al.*, 2011). Thus, enriching the early social environment through manipulations which increase maternal care can induce epigenetic modifications which alter neurodevelopmental outcomes.

5. Human studies of the epigenetic impact of childhood adversity

Though translating the findings of animal studies to better understand the impact of social experiences in humans is challenging, there is increasing evidence that epigenetic variation can be observed among individuals that have experienced childhood adversity. For example, analysis of postmortem human

brain tissue suggests that individuals with a history of childhood abuse have decreased hippocampal *GR* mRNA associated with increased DNA methylation within the *GR* 1F promoter region when compared to nonabused subjects (McGowan *et al.*, 2009). In blood samples from orphans raised in institutions, genome-wide levels of DNA methylation have been found to be altered, with institution-raised children having overall higher levels of CpG methylation compared to children raised by their biological parents (Naumova *et al.*, 2011). The use of blood biomarkers in human studies of the epigenetic impact of social experiences may allow for the application of an epigenetic perspective to many critical questions regarding the timing, specificity, and stability of the effects of social environments on human development.

B. Adult social stress

Neurobiological and behavioral plasticity is not limited to infancy/early childhood and can certainly be observed in adulthood. In rodents, this plasticity can also be observed within epigenetic pathways. Though social interactions are important for normal development, and long-term social isolation can impair emotional behavior (Heidbreder *et al.*, 2000), the quality of those social encounters will be an important predictor of long-term health outcomes. In adult rodents, exposure to aggressive social interactions can have a significant impact on social behavior and induce depressive-like behaviors. The "social defeat" model has been used to induce these effects (Martinez *et al.*, 2002; Tamashiro *et al.*, 2005) and consists of placing an "intruder" into the cage of a "resident," typically a larger, dominant adult male (Miczek, 1979). In this paradigm, the intruder is exposed to repeated aggressive encounters and is defeated in these interactions. As is the case for early life adversity, this manipulation of the adult social environment results in reduced locomotion, social avoidance, and increased HPA activity (Blanchard *et al.*, 1993; Keeney and Hogg, 1999; Meerlo *et al.*, 1996; Raab *et al.*, 1986). Among socially defeated male mice, there are reduced hippocampal levels of *BDNF* that can be observed for a month following the experience of defeat (Tsankova *et al.*, 2006). Within the *BDNF* III and IV promoter regions, there is increased histone H3K27 dimethylation in socially defeated males which may account for the reduced *BDNF* expression. Histone deacetylase (*HDAC5*) mRNA levels are also found to be decreased in socially defeated males (Tsankova *et al.*, 2006). Differential levels of histone H3K27 dimethylation are also found across the genome within the nucleus accumbens (NAc), in response to both chronic social defeat and prolonged adult social isolation (Wilkinson *et al.*, 2009). Analysis of histone acetylation in the NAc indicates that H3K14 acetylation is initially decreased and then increased following chronic social defeat associated with decreases in *HDAC2*

levels. Increased H3 acetylation in the hippocampus and infralimbic medial prefrontal cortex has also been observed in socially defeated rats (Hinwood *et al.*, 2011; Hollis *et al.*, 2010).

Individual differences in the effects of social defeat have been observed at both the behavioral and epigenetic level of analysis (see Fig. 2.3). Though social defeat has been found to induce long-term reductions in social approach behavior, there are some individuals that display resilience to this stressor (Wilkinson *et al.*, 2009). Stress-susceptible mice have been found to have increased levels of *CRF* mRNA in the PVN and decreased CpG methylation within the *CRF* gene promoter, whereas stress-resilient mice were found to have no changes in *CRF* mRNA or DNA methylation of this gene (Elliott *et al.*, 2010). In rats, individual differences in response to novelty predict differential epigenetic effects following exposure to social defeat. Among rats that are highly exploratory in a novel environment, social defeat results in decreased H3K14 acetylation, whereas among rats that engage in low levels of exploratory behavior, social defeat is associated with increased H3K14 acetylation (Hollis *et al.*, 2011). Overall, these studies highlight the complex interactions between stress susceptibility, social experiences, and epigenetic pathways which will certainly be an important consideration in the study of epigenetic effects in humans.

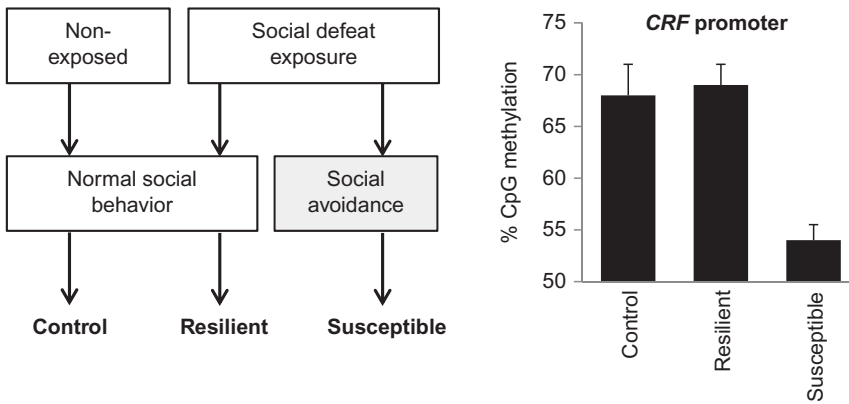

Figure 2.3. Summary of the effects of adult social defeat in mice on DNA methylation within the corticotropin-releasing factor (*CRF*) gene. The experience of social defeat results in lasting neurobiological and behavioral changes. In particular, this experience of social adversity typically leads to reduced social approach behavior (susceptible). However, some individuals do not exhibit impairments in social behavior following the experience of social defeat (resilient). Analysis of the degree of CpG methylation within the *CRF* gene promoter suggests that both nonexposed (control) and resilient mice have elevated levels of *CRF* CpG methylation, whereas susceptible mice have reduced *CRF* CpG methylation.

III. ROLE OF EPIGENETICS IN SHAPING SOCIAL BEHAVIOR

The previous section highlighted the growing evidence for the influence of social experiences on epigenetic mechanisms such as DNA methylation and histone modifications. In many cases, these epigenetic effects have consequences for social behavior. For example, the experience of Low LG or postnatal abuse leads to the emergence of these phenotypes in exposed individuals (Champagne, 2008; Roth *et al.*, 2009). In the case of social defeat, epigenetic modifications may result in the sustained inhibition of social behavior among exposed individuals (Berton *et al.*, 2006). Beyond these examples, there is increasing evidence for the role of epigenetic mechanisms in shaping social behavior derived from studies in which epigenetic pathways are directly manipulated or through modification of epigenetic pathways via nonsocial environmental cues which trigger sexual differentiation.

A. Epigenetic dysregulation and social behavior

The increased incidence of autism and other disorders characterized by social deficits has led to the development of diverse research approaches exploring both the genetic and epigenetic etiological pathways that account for disorders of the social brain. Genetic association studies have indicated a link between autism and mutations in the genes encoding the methyl-binding protein MeCP2 (Cukier *et al.*, 2010; Schanen, 2006), the histone deacetylase HDAC4 (Williams *et al.*, 2010), an H3K9 methyltransferase (EHMT1) (Balemans *et al.*, 2010; Kleefstra *et al.*, 2010), and the H3K4 demethylase JARID1C (Adegbola *et al.*, 2008). These genetic effects have consequences for epigenetic chromatin remodeling in the autistic brain. Within neuronal cell populations in the prefrontal cortex, abnormalities in the pattern of H3K4 trimethylation have been detected in the postmortem brains of autistic individuals and these epigenetic modifications lead to altered mRNA levels of genes implicated in neurodevelopment (Shulha *et al.*, 2011). Similarly, in mice, autism-like deficits in cognition and social behavior can be induced through targeted deletion of genes involved in epigenetic pathways. Mutation of the *EHMT1* (histone-lysine *N*-methyltransferase) gene has been found to induce reductions in exploratory behavior and deficits in social play and suppress preference for social novelty in mice (Balemans *et al.*, 2010), and in humans, mutation of this gene is associated with Kleefstra syndrome—a developmental disorder with autistic-like features (Kleefstra *et al.*, 2010). In humans, mutations within the *MeCP2* gene are associated with the development of Rett syndrome, characterized by cognitive decline in infancy and the development of stereotyped behaviors (Amir *et al.*, 1999; Hagberg *et al.*, 1983). Disruption to this gene in mice has indicated a role for MeCP2 in social behavior (Moretti and Zoghbi, 2006), and impairments in

the functioning of *MeCP2* are associated with elevated H3 acetylation in the cortex (Shahbazian *et al.*, 2002). The importance of histone hyperacetylation in the etiology of social deficits has also been demonstrated using the HDAC inhibitor valproic acid. In mice, prenatal treatment with valproic acid has been found to induce cognitive impairments, increased anxiety-like behavior, and deficits in social interactions (Kataoka *et al.*, 2011). The hyperacetylation induced by valproic acid may lead to increased apoptosis in the developing prefrontal cortex with implications for cortical layering. Thus, epigenetic dysregulation, induced through genetic or pharmacologic factors, may have implications for a broad range of neurodevelopmental abnormalities.

B. Sexual differentiation

Hormonal signals occurring during development play a critical role in the sexual differentiation of the brain with implications for behavioral patterns exhibited by males and females. Across species, social play behavior has been found to be a sexually dimorphic behavior, with males typically displaying elevated levels of social play—particularly "rough-and-tumble" social interactions (Auger *et al.*, 2011; Meaney, 1989; Paukner and Suomi, 2008). Thus, sexual differentiation is a predictor of social behavior, and there is increasing evidence for the role of epigenetic mechanisms in the developmental process of this differentiation (Auger *et al.*, 2011; McCarthy *et al.*, 2009). The MPOA is a sexually dimorphic brain region that is typically larger in males than in females (Gorski *et al.*, 1978), and within this region, females have been found to have elevated levels of *ERα* mRNA and protein compared to males (Kurian *et al.*, 2010). The reduced transcription of *ERα* in males may be attributed to elevated levels of DNA methylation in the 1B promoter region of the *ERα* gene. However, if female rat pups are treated with estradiol on postnatal day 2, there is a decrease in *ERα* mRNA and this hormone-induced change in gene expression is associated with increased CpG methylation within the *ERα* gene promoter (Kurian *et al.*, 2010). Females can also become masculinized (decreased preoptic *ERα* mRNA, increased *ERα* 1B promoter methylation) by receiving licking-like tactile stimulation (using a paintbrush) during the postnatal period (Kurian *et al.*, 2010). Maternal LG has been observed to be directed more frequently to male pups during postnatal mother–infant interactions and may be an important behavioral pathway in the development of sexual differentiation (Moore, 1984; Moore and Morelli, 1979). Reductions in sexual dimorphism of neurobiological and behavioral measures has also been observed following exposure to endocrine disruptors, such as bisphenol A (BPA), and there is increasing evidence for the modification of epigenetic pathways by these compounds (Kundakovic and Champagne, 2011). BPA has been found to induce sex-specific effects on social behavior in juvenile mice, possibly through altered expression of the DNA methyltransferases

DNMT1 and *DNMT3a* (Wolstenholme *et al.*, 2011). Overall, these studies suggest that exploring the epigenetic basis of sexual differentiation and the processes (behavioral and hormonal) which alter differentiation may provide insights into the role of epigenetic mechanisms in shaping the social brain.

IV. CONCLUSIONS AND FUTURE DIRECTIONS

Epigenetic mechanisms play a critical role in development and may serve both to shape development in response to social experiences and to induce variation in social behavior. Thus, these molecular pathways illustrate the dynamic interactions between genes and environments that account for the origins of our unique neurodevelopmental and behavioral trajectories. The plasticity of DNA methylation and posttranslational histone modifications in response to both postnatal and adult social experiences suggests that these mechanisms may have evolved to allow organisms to adapt to changing environmental conditions. The persistence of behavioral, neurobiological, and epigenetic variation across generations that has been observed following manipulation of the social environment (Champagne, 2008; Franklin *et al.*, 2010; Roth *et al.*, 2009) may have implications for our notion of "inheritance" and the factors that contribute to the similarity between ancestors and descendants. Thus, our perspective on the factors driving both individual differences and the heritability of individual variation is increasingly combining both genetic and epigenetic pathways.

The field of behavioral epigenetics is rapidly expanding, and analysis of epigenetic mechanisms is being increasingly applied across species and taxa (Kucharski *et al.*, 2008; Meyer, 2011; Roy *et al.*, 2010). The incorporation of diverse species with unique life history characteristics will most certainly provide novel insights into both the convergent and divergent routes through which epigenetic plasticity drives phenotypic diversity. An important consideration within these studies will be the complex interactions between transcriptional, posttranscriptional, and posttranslational epigenetic modifications that are involved in the dynamic process of gene regulation. In addition, understanding the genetic basis of epigenetic variation, which is being increasingly explored in the context of social deficits in behavior, is a critical step in determining the evolutionary basis of epigenetic variation.

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