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Epigenetic Mechanisms and the Developing Brain

Bridging the Nature–Nurture Divide

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Abstract

Epigenetic mechanisms are critical to the developing brain. This chapter reviews epigenetic mechanisms, their involvement in the processes of brain development, and the literature suggesting that epigenetic mechanisms may account for the enduring effects of environmental factors on the brain and behavior in human development. Epigenetic factors guide the expression of the genome in response to the intrinsic signals inherent to the processes of embryogenesis, neurogenesis, cell migration, synaptic transmission, and the timing of developmental windows. Moreover, evidence suggests that epigenetic regulators may account for the embedding of early social experiences within neurobiology. These early modifications to the epigenetic code are hypothesized to have consequences for developing neural structures and function. Epigenetic changes might also channel or moderate the effects of genetic variation on emotional and cognitive processes, and psychiatric conditions. Thus, the study of the epigenetic consequences of early-life environments may shed light on the biological pathways of environmentally induced risk.

Introduction

Epigenetics refers to the processes that allow identical DNA sequences to give rise to a diversity of cells. Waddington first reasoned that there must be contextual factors acting "upon" (the Greek root meaning of "epi") the genome to guide developmental processes (Van Speybroeck 2002). Today, the elucidation of these mechanisms has been achieved in part through the study of epigenetics, more recently defined as the structural adaptation of chromatin in a manner that alters or regulates the activity states of genes without modifying the genetic code itself (Bird 2007; Meaney 2010). Hence, the expression of the

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genome (i.e., DNA and encoded nucleotide sequences) is better understood accompanied by knowledge of the epigenome, inclusive of the structures and molecules affecting the packaging of chromatin and activity states of DNA, which can be both the cause and the consequence of the transcription of genomic material (Jones et al. 2013).

Beyond unraveling the cellular mechanics behind the selective transcription of gene sequences, epigenetics has provided insight as to how developmental signals, ranging from intracellular to external stimulation, might impact the expression of the genome. Epigenetic processes are essential to the developing biology of the body and brain: in conjunction with programs initiated by transcription factors, epigenetic marks determine the stable histological fate of cells, yet also allow the organism's characteristics to develop and adapt appropriately to environmental context. Because the epigenome is plastic and modifiable, epigenetic marks not only alter gene expression, they may also carry the vestiges of developmental history. In this way, epigenetic patterns may serve as a link between the interplay of genotype, developmental context, and functional biology.

In the following, we begin with a brief review of epigenetic mechanisms and then describe how epigenetic modifications channel gene expression in the formation and strengthening of neural connections over the course of development. Specifically, we review detailed evidence that (a) epigenetic modifications are critical regulators in normative neurodevelopmental processes, including the organization of the nervous system in embryogenesis, neurogenesis, neuronal migration, and synaptic transmission and plasticity, and (b) epigenetic modulation might account for how early environmental experiences mold the developing brain in humans.

Epigenetic Mechanisms

Our focus here is on epigenetic mechanisms that are involved in the regulation of transcriptional potential. Some of these mechanisms act at the level of chromatin, the packaging of DNA within chromosomes which allows ~2 meters of DNA in each cell to be condensed within the cell nucleus. The basic unit of chromatin is the nucleosome, comprised of 147 base pairs of DNA wrapped around a histone protein octamer, with each cell containing 3×10^7 nucleosomes, connected together by linker DNA. Some epigenetic "marks" or chemical tags affect how loosely or tightly chromatin is wound around the nucleosomes, and consequently the degree of physical access of DNA to transcriptional machinery. Other epigenetic mechanisms act directly at the level of DNA structure, while others activate or inhibit transcription factor proteins, affecting their ability to enhance or inhibit expression. Finally, epigenetic mechanisms may involve noncoding RNA, which regulates gene expression at the transcriptional or posttranscriptional level.

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DNA methylation is a well-characterized epigenetic mark, most popularly studied as the covalent, chemical modification to a cytosine base adjacent to a guanine base (i.e., a CpG dinucleotide). CpG islands are areas of the genome with high CpG content (Illingworth and Bird 2009). Rather than a random distribution across the genome, CpG islands are found proximal to 70% of gene promoters, the noncoding sequences preceding coding DNA regions where transcription elements bind to regulate gene activity (Saxonov et al. 2006; Illingworth and Bird 2009). Higher DNA methylation in promoter regions (which often means within CpG islands) is linked to lowered gene expression, whereas in the gene body or coding region, methylation is more often associated with enhanced gene expression (Jones 2012). This trend generally holds true when analyzing genes within individuals; however, in cross-individual comparisons of single genes, the relationship between DNA methylation and transcription is more complicated and may relate to a negative, positive, or null relationship with gene expression (Lam et al. 2012; Gutierrez-Arcelus et al. 2013; Klengel et al. 2014).

DNA methylation is not restricted to CpGs; non-CpG methylation (CpH; H = A, T, or C) patterns in the mammalian brain demonstrate conservation across species. CpH methylation, unlike the majority of CpGs, is established *de novo* during neuronal maturation, suggesting it could be a key regulatory mechanism for the neuronal genome. CpH methylation, like CpG methylation, can repress transcription *in vitro* and is bound by MECP2 (a protein essential to neuron function) *in vivo* (Guo et al. 2013). Finally, the abundance of CpH in the frontal cortex of the mammalian brain, with its levels inversely related to gene transcription, further supports functional relevance (Lister et al. 2013). It has also been shown that DNA can be actively demethylated via the hydroxymethylation of CpGs (Guibert and Weber 2013; Jones et al. 2013), which is critical for neuronal differentiation and function (Santiago et al. 2014). Thus, DNA methylation and demethylation, both within CpGs and at CpH sites, are avenues of epigenetic regulation.

Histone modifications describe posttranslational alterations to the histone proteins of nucleosomes: H2A, H2B, H3, and H4. The structure of nucleosomes and chromatin are affected by a number of modifications (e.g., acetylation, phosphorylation, methylation) that can occur at over 100 sites of protein N-terminal tails, as well as across histone core domains (Mersfelder and Parthun 2006; Bridi and Abel 2013). These numerous modifications can influence nucleosome stability and positioning, ultimately affecting the state of chromatin and accessibility of particular genes (Venkatesh and Workman 2015). It has been suggested that specific combinations of histone modifications, referred to as the "histone code," correspond to particular transcriptional states (Strahl and Allis 2000; for discussion of the debate surrounding the histone code hypothesis, see Rando 2012). The enzymes that modify histones occur together within regulatory complexes, guiding the co-occurrence of synergistic histone marks necessary for transcriptional outcomes (Day and Sweatt 2011). In addition to

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targeting specific genes, histone marks can localize within a gene to regulate specific locations within the exon–intron structure, providing selective gene readout (for further details, see Day and Sweatt 2011). Finally, the canonical histone proteins mentioned above can be replaced by histone variants independent of replication, and these variants can result in differentiation of chromatin with epigenetic consequences (Henikoff and Smith 2015). Taken together, modifications to histone proteins and histone variants offer multiple levels of complexity in terms of epigenetic regulation.

Noncoding RNA molecules (ncRNA), including long RNA, microRNA (miRNA), small interfering RNA, and small nuclear RNA, serve additionally as epigenetic marks with effects on activation, repression, and interference with expression. The majority of the mammalian genome is transcribed into ncRNAs (molecules that do not encode for proteins) and comprise an additional layer of internal cellular information (Mattick and Makunin 2006). Gene expression is controlled by ncRNAs at multiple levels (e.g., chromatin architecture, epigenetic memory, splicing, transcription and translation) for the normal processes of physiology and development. For instance, miRNAs bind to the 3′ untranslated messenger RNA regions or mRNA coding sequence, degrading mRNA or regulating its expression through translation (Day and Sweatt 2011). Composed of 20–25 nucleotides of noncoding RNA, miRNAs are particularly relevant, as they control the expression of the majority of genes in the genome. As will be reviewed in more detail below, miRNA regulation is a key mechanism for development and plasticity of the nervous system.

Two additional means of epigenetic regulation are noteworthy and relevant to this discussion. First, the regulation of the expression of specific isoforms of a protein arises through a process of alternative splicing of exons. Epigenetic marks, such as histone modifications (Bridi and Abel 2013) or DNA methylation at exon–intron junctions (Jones 2012), can regulate alternative splicing, thus leading to different splice variants of the same gene, which have different functions and affinities for effector proteins. For instance, modifications to histone proteins can affect the recruitment of splicing regulators, and thus the protein product outcome of splicing.

Second, genomic imprinting describes the acquirement of epigenetic modifications to DNA or histone proteins (discussed above) from one of the parental gametes in a manner that biases the expression toward only one gene copy (Perez et al. 2016). Genomic imprinting occurs in at least ~50 human genes (Ishida and Moore 2013), and these parental expression biases have important functional significance for both imprinted and nonimprinted genes within regulatory gene networks (Perez et al. 2016). Although imprinting was originally identified as the silencing of one parental allele and consequent monoallelic expression of the second parental allele, the prominence of parental allelic bias on a continuum from weak to monoallelic expression, rather than solely an all or none monoallelic effect, has since been identified (Perez et al. 2016). Whether monoalleic expression or some level of parental bias of an imprinted

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gene occurs is complex and often depends on tissue or developmental stage (Martinez et al. 2014).

In summary, epigenetic machinery can be thought of as an overlay to the genome, providing the flexible and specific gene readouts required for the complex patterns of expression that take place in the development of the organism.

Neurodevelopment

Given the connection between epigenetic modification and gene expression, it is intuitive that epigenetic mechanisms may play a role in bridging from the genetic code to complex neurodevelopmental processes. In neurodevelopment, cells must be able to express or repress sets of genes to ensure that cells differentiate and migrate to proper locations, and that synaptic connections form and adjust. Consistently, epigenetic shifts occur simultaneously with normative phases of brain development and plasticity in mammalian neurodevelopment. Below, we describe the role of epigenetic mechanisms in shaping embryogenesis, neurogenesis and migration, neuronal plasticity, and critical windows of development in which the fate of neurons and circuitries may be especially sensitive to the effects of external stimulation. The focus is primarily on DNA methylation and ncRNA epigenetic mechanisms, as these are extensively studied, but we also highlight other processes reviewed above when relevant.

Embryogenesis

In humans, embryogenesis occurs during the first eight weeks of development, in which a fertilized egg is transformed to a multilevel body plan. Although epigenetic mechanisms are highly involved in guiding the processes required for the differentiation of cells into the various types across the body and brain, here we focus on the differentiation of cells in the central nervous system.

Mammalian neurodevelopment involves a coordinated sequence of genomic methylation and demethylation in the creation of functionally distinct neuron and glia populations (Wu and Zhang 2014). In human embryogenesis, two waves of genome-wide DNA demethylation occur: the paternal genome is demethylated a few hours post fertilization, and the maternal genome is demethylated after the two-cell embryo stage (Haaf 2006). This global DNA demethylation is followed by epigenetic reprogramming, in which epigenetic factors intersect with transcription factors to assist the differentiation of cells into at least 200 different histological types through the calibration of ~20–25k protein-coding genes.

During this phase of epigenetic reprogramming, the patterns of DNA methylation which emerge during cell differentiation are reliably reproduced in daughter cells. The epigenetic patterns responsible for stable expression

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profiles specific to cell type, as well as random defects in epigenetic marks, are maintained across the human lifespan. The mitotic replications of differentiated cells are subject to stochastic errors, the rate of which is higher for epigenetic marks relative to DNA replication. In humans, it is very difficult to parse marks that stem from random developmental processes versus those that arise due to important cellular or environmental signals with potential functional consequences, though each of these sources may lead to stable epigenetic differences between individuals.

During embryogenesis, the differentiation of neural stem cells into neurons and glia requires the induction of multiple transcription factors that activate cell type-specific transcriptional programs. This process is highly regulated by imprinted genes. For instance, a maternally expressed miRNA cluster promotes the shift from neural stem cell proliferation to differentiation and migration (Rago et al. 2014) and neural stem cells express a paternally expressed zinc finger protein, PLAGL1 to express a maternally imprinted gene promoting the arrest of neural stem cell cycle, and subsequent differentiation (Hoffmann et al. 2014). Imprinted genes are required for higher-level specific structures as well, such as the differentiation of GABAergic interneurons and Golgi cells in the cerebellum (Chung et al. 2011) and midbrain dopaminergic neurons (Hoekstra et al. 2013).

In summary, the transition of a single cell to the high-level organization of the emerging mammalian brain is highly regulated by epigenetic processes. The creation and placement of new cells, discussed in the following sections, involves a similar set of epigenetic machinery.

Neurogenesis

Neurogenesis is the process by which neural stem or progenitor cells generate new neurons during embryonic and perinatal development. Neurogenesis also occurs in adulthood within the subventricular zone of the lateral ventricles as well as within the subgranular zone of the dendate gyrus (Ming and Song 2011). Epigenetic mechanisms guide neurogenesis during early development through coordinated responses to extracellular signals, which modulate the expression of transcription regulators controlling cell proliferation, cell-type specification, and the differentiation of neural progenitor cells. In adults, epigenetic modifications remain critical for maintaining neural progenitor cells and guiding their fate through spatial and temporal expression of transcription regulators (Yao et al. 2016).

Multiple epigenetic modulators are required for neurogenesis. Protein complexes that orchestrate histone methylation and demethylation are in tight control of gene expression during neurogenesis. Transcription factors that bind to DNA to regulate neurogenesis are guided to proper sequences by the presence and absence of DNA methylation marks (Wang et al. 2016). During embryogenesis, miRNA miR-19 is responsible for neuronal progenitor cell

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proliferation and radial glial cell expansion. Long RNAs (a type of noncoding RNA mentioned above possessing over 200 nucleotides) recruit transcription factors that bind to intergenic regions to modulate the expression of key homeobox genes (Yao et al. 2016). These long RNA molecules are also essential for the neurogenesis of GABAergic interneurons in the postnatal hippocampus (Yao et al. 2016). Hence, epigenetic mechanisms are critical regulators of neurogenesis across developmental time.

Neuronal Migration

Neuronal migration plays a critical role in establishing cell identity and functional connectivity in the developing brain, and involves key epigenetic modulators. For instance, through the regulation of transcriptional programs, histone methyltransferase Ezh2 controls the topographic neuronal guidance and connectivity of the pontine nuclei, which serve as the main relay point between neocortex and cerebellum (Di Meglio et al. 2013). Epigenetic mechanisms also inhibit neuronal migration once neurons have reached their destination. For example, cortical neuron migration is inhibited when DCX is silenced by maternally expressed miR134 (Gaughwin et al. 2011). Finally, much of the guidance of migration is dependent on neuronal activity, which shapes expression through epigenetic pathways. Maternally expressed KCNK9 controls resting potentials and excitability of neurons, and maternally transmitted mutations in this gene are responsible for impaired neuronal migration and maturation of dendrites in Birk-Barel syndrome (Bando et al. 2014). To conclude, epigenetic modulators guide the proper migration of neurons in the developing brain as well as, ultimately, the establishment of functional circuitries.

Synaptic Transmission and Plasticity

Consistent with a role for epigenetic regulation in plasticity processes, there is substantial evidence for their involvement in activity-dependent processes critical to the formation and plasticity of neural connections. Empirical investigations in animal models have exemplified how various markers, including histone modifications and DNA methylation, may orchestrate sets of changes to specific signals and behavioral experiences, and that these changes are necessary for plasticity. These changes are relevant from the level of individual cell adaptation to the multicellular plasticity events underlying the formation and consolidation of memory.

Brain-specific miRNAs, which are transiently and locally expressed in dendrites and responsive to neuronal activity, have been shown to mediate the regulation of gene functions that contribute to learning and memory (Bredy et al. 2011). A number of key miRNA proteins are expressed in dendrites and regulate spine formation and synaptic plasticity within hippocampal neurons

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via regulation of the expression of brain-derived neurotrophic factor (Ye et al. 2016). It has been suggested that miRNAs contribute to neural plasticity and memory by regulating dendrite morphogenesis in early development and by fine-tuning gene function via translation regulation within synapses (Bredy et al. 2011).

The proper wiring of neuronal circuits through regulation of synaptic transmission is highly dependent on genomic imprinting mechanisms, as imprinted genes are involved in neuronal transmission, and on activity-dependent alterations of neuronal excitability states. Maternally expressed KCNK9 regulates membrane-resting potential and changes in firing patterns (Musset et al. 2006; Brickley et al. 2007). Imprinting mechanisms also regulate presynaptic vesicles, important for the strength of postsynaptic signals, and long-term potentiation (LTP) of NMDA and AMPA glutamate receptors, which are mechanisms of excitatory synaptic plasticity (Fleming and England 2010). In addition, imprinted genes modulate the maintenance of excitatory–inhibitory balance within neuronal circuits (Wallace et al. 2012).

Histone modifications are responsible for the transcriptional flexibility observed in the cellular events of memory formation. Epigenetic networks regulate short- and long-term changes in the chromatin environment, modifications which are required for memory acquisition and synaptic plasticity in the cortex, hippocampus, striatum, and amygdala (Bridi and Abel 2013). Histone acetylation is important for reconsolidation and extinction; phosphorylation is involved in the transcriptional effects triggered by external stimulation; and histone methylation has been linked to the activation and repression of the protein complexes that regulate histone acetylation in plasticity processes (Bridi and Abel 2013; Ciccarelli and Giustetto 2014). For instance, interference with the molecular mechanisms regulating histone acetylation alters associative learning and the LTP cellular correlates of memory (for a review, see Bridi and Abel 2013).

It also appears that histone variants could be key players in guiding neuroplasticity processes. A variant of histone H2A, H2A.Z, actively replaces H2A following fear conditioning in the hippocampus and cortex. H2A.Z appears to mediate gene expression in these brain areas in a manner that inhibits the formation of memory (Zovkic et al. 2014). Furthermore, a recent study in mice reported H2A.Z deposited at the promoter of activity-dependent genes is responsible for triggering their deactivation and interfering with dendritic pruning (Yang et al. 2016). Essentially, through its regulation of activity-dependent transcription, the presence of this histone variant has lasting implications for the patterning of dendrites and the coding of sensorimotor information in the brain. Histone variants, then, may be an important and to date understudied epigenetic mechanism relevant to sensorimotor and cognitive processes in key brain regions.

DNA methylation also plays a role in synaptic plasticity (Baker-Andresen et al. 2013). One study observed genome-wide CpG methylation before and after

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neuronal activation in the adult mammalian brain, reporting dynamic changes in DNA methylation (Guo et al. 2011). These alterations in methylation were prevalent in areas of lower CpG density and occurred within genes involved in brain development and neuronal plasticity. The authors concluded that there may be a key role of the DNA methylome in activity-dependent epigenetic regulation of neuroplasticity, which may concentrate around areas of low CpG density rather than CpG islands (Guo et al. 2011).

DNA methylation within various brain regions is also relevant to memory formation and consolidation. DNA methyltransferase (DNMT) enzyme activity regulates the induction of hippocampal LTP: DNMT expression is significantly enhanced within the hippocampus after contextual learning takes place, and blocking its activity in the hippocampus disrupts the formation of associative memory (Yu et al. 2011b). The brain-derived neurotrophic factor (BDNF) gene, which has been linked to the persistence of fear memories, shows dynamic changes in DNA methylation in the hippocampus in response to fear conditioning (Lubin et al. 2008). Moreover, changes in BDNF methylation are reversed with the application of a DNMT inhibitor and NMDA receptor blocker, both of which correspond with impaired memory formation. DNA methylation and histone acetylation within the amygdala have also been shown to support learning and memory processes. Finally, the cortex has been targeted in the study of epigenetic regulators of LTP, as the hippocampus and amygdala have been implicated in associative learning and memory, but are not essential for long-term memory. Studies focused on the cortex have shown that contextual fear conditioning has robust and enduring alterations in DNA methylation in the anterior cingulate cortex, at least 30 days following conditioning. Long-lasting memory can be reversed by inhibiting DNMT in the anterior cingulate cortex, suggesting the ongoing relevance of DNA methylation in the cortex for memory stabilization (Day and Sweatt 2011). Thus, DNA methylation appears to modulate learning and memory within multiple areas of the brain involved in associative and long-term memory formation.

In summary, epigenetic mechanisms have been implicated as part of the activity-dependent machinery responsible for the formation and molding of synaptic connections. Because neuronal activity is the critical mechanism by which external stimulation modulates neural circuits, this suggests that epigenetic modulators are indeed a critical factor in bridging signals from the external environment to functional neurobiology.

Epigenetic Regulation of Critical Periods of Plasticity

Early development marks a phase of heightened plasticity and malleability to contextual surroundings, encompassing critical periods of brain development. A critical period is a window of enhanced developmental plasticity in which experiences have accentuated, irreversible effects on neural circuits

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(Fox et al. 2010). For instance, there is a critical period for exposure to linguistic experience in humans—including variation in sounds, language, and practice producing language—which is required during an early developmental window if humans are to develop the neural circuitries underlying the capacity for speech (Kuhl 2004). Critical periods of brain development also render infants and children more susceptible to the effects of the social environment. For example, in a study of Romanian orphans randomly assigned to foster homes at various ages, it was observed that children placed prior to the age of 2 years demonstrated substantial gains in cognitive and emotional outcomes relative to children who remained at the orphanages (an environment of extremely high social deprivation) until later ages (Zeanah et al. 2011).

These critical periods are initiated, guided, and terminated by epigenetic molecular events affecting the expression of neuroregulatory genes (Fagiolini et al. 2009). The epigenetic molecular substrates of these developmental windows, serving as triggers and breaks, can initiate and constrain brain plasticity (Takesian and Hensch 2013; Werker and Hensch 2015). Advances suggest that the brain's default state is plastic, with the solidification of cell functions and neural networks requiring a timed and synchronized suppression of this plasticity. For example, the end of the critical period for acquisition of ocular dominance involves a downregulation of vision-dependent acetylation and phosphorylation of histones (Putignano et al. 2007). In adults, the removal or inhibition of histone deacetylases (the enzymes that remove acetyl groups from histones) reactivates plasticity in the primary visual cortex via changes of chromatin organization that enhance the accessibility to transcription (Lennartsson et al. 2015). Thus, epigenetic molecular mechanisms not only drive ongoing plasticity processes, they open and close critical windows in which brain development is particularly sensitive to incoming signals.

Summary

The architecture of the brain is established and modified by a continuous series of dynamic interactions between the genome and the developmental signals which modulate its expression via epigenetic machinery. Chromatin structure is dynamic and incorporates hundreds of signals from the cell surface to achieve the coordinated transcriptional outcomes that guide each of these neurodevelopmental steps. Epigenetic marks upon chromatin and DNA integrate these signals, creating the enduring signatures that determine cell fate, guide neurogenesis and migration, synaptic plasticity, and even the opening and closing of developmental windows. In addition, epigenetic mechanisms bridge external environmental signals to neurodevelopment within critical periods of plasticity, as will next be explored.

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Neurobiological Embedding of the External Environment during Critical Periods

Beyond intrinsic cellular signals, epigenetic machinery appears to be responsive to external environmental cues and possibly responsible for encoding these signals within developing neural circuitries. Since the brain is highly plastic during early critical windows, and associations between stressful early experiences and later developmental outcomes in humans have been observed, researchers have sought to explore the possibility that epigenetic mechanisms account for the impact of early experiences on the developing brain and subsequent psychological outcomes.

Here we briefly discuss the animal work that spearheaded investigations of epigenetic modulators in relation to human early social experiences. We then explore the existing human literature that links early environments to epigenetic markers and neural structure and function.

Animal Research: Potential Link between Early Social Environments and Epigenetic Machinery

The potential role of epigenetic marks as a biological consequence of early forms of social environmental adversity was first studied by leveraging natural variation in maternal behavior in rats (Weaver et al. 2004). Epigenetic markers in stress-related areas of the brain were compared between rat pups that experienced low versus high maternal care (measured by frequency and duration of licking and grooming). Low levels of care corresponded with upregulated hypopituitary adrenal axis (HPA) reactivity through an epigenetic mechanism: pups reared in low-care early environments demonstrated increased DNA methylation and decreased histone acetylation of the glucocorticoid receptor (GR) gene *NR3C1*, which ultimately was associated with diminished expression of GR and upregulation of CRH secretion and HPA activity. Although this seminal study sparked a large body of research exploring methylation of the GR promotor, it is important to note that efforts to replicate the original finding in terms of direction and effect size are still underway (Pan et al. 2014; for a discussion, see Boyce and Kobor 2015).

Further investigations in mice and rats reported links between maternal care, maternal separations, and communal rearing and altered methylation and histone acetylation in the hippocampus, as well as links between maternal care and epigenetic markers within the hypothalamus, amygdala, pituitary gland, and prefrontal cortex (for a review, see Kundakovic and Champagne 2015). There is additional evidence in rhesus macaques that rearing conditions and social rank associate with subsequent epigenetic variation within blood and prefrontal cortex (PFC) cells, as well as gene regulation (Kinnally et al. 2011; Provençal et al. 2012; Tung et al. 2012). These primarily experimental findings

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suggest a causal link between early social experiences and subsequent epigenetic profiles within critical regions of the brain. Next, we discuss the evidence for this connection in human studies utilizing both peripheral and central tissues.

Human Research on Association between Early Environments and Epigenetic Markers

Although limited in the ability to distinguish cause from correlation, human work on early environmental factors and DNA methylation has demonstrated potential biological signatures of early adverse experiences. Links to methylation patterns may suggest that early social experiences had effects on neurodevelopmental processes, although this cannot be determined. Beyond the correlational nature of human studies, another noteworthy issue is the typical use of peripheral tissues (including blood, saliva and buccal epithelial cells) rather than brain tissue to obtain epigenetic profiles. Brain tissue is obtained postmortem or during a required surgical procedure, and thus is not a viable option for large-scale studies. Because different tissues show distinctive epigenetic patterns, and variation in methylation is most largely driven by cell-type composition (Jaffe and Irizarry 2014; Farré et al. 2015), the biological variation associated with the cell type of the collected tissue must be taken into consideration to identify any meaningful interindividual differences in methylation profiles. Finally, it is worth noting that although the focus on DNA methylation in this literature is typically tied to an interest in the expression of genes in the developing brain, the relationship between epigenetic marks and gene expression is complex. DNA methylation might be the cause or the consequence of gene expression, which can depend on the direction of a DNA methylation change, location relative to the gene, and specific function of CpGs. With these caveats in mind, let us look at the existing literature linking prenatal and postnatal environments to patterns of DNA methylation in humans.

Prenatal Environment

Substantial correlational work in humans has linked prenatal experiences to differential methylation patterns at later developmental periods. This suggests that epigenetic marks are involved in potential prenatal programming of subsequent phenotypes. Most of this work has targeted candidate genes. Here we briefly highlight interesting findings, but refer the reader to Cao-Lei et al. (2016) for a more detailed review.

Work in human prenatal exposures is generally limited to maternal reports of mood and exposures encountered during pregnancy. Much of the

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candidate gene work has focused on *NR3C1*, the gene encoding the glucocorticoid receptor, which was initially implicated in experience-dependent epigenetic regulation of the stress response in animal work (for a review, see Turecki et al. 2016). In a number of studies, prenatal stress in the form of maternal depression, exposure to intimate parent violence, psychological well-being, and extreme trauma was associated with methylation of *NR3C1* in offspring at later developmental periods (Oberlander et al. 2008; Radtke et al. 2011; Hompes et al. 2013; Perroud et al. 2014). Moreover, the link between intimate partner violence and *NR3C1* methylation status in children was specific to the prenatal period, as no association was found for maternal stress prior or subsequent to pregnancy (Radtke et al. 2011). Notably, this popularly targeted promoter region is largely invariable, with very low levels of DNA methylation across individuals. Consistently, the effect sizes reported are extremely small. Perhaps more problematic, the majority of candidate gene methylation studies do not adjust for cell-type composition of samples, the largest contributor to variability in DNA methylation. These pitfalls warrant interpreting these findings, as well as additional candidate findings targeting invariable promoters discussed below, with a healthy dose of caution.

DNA methylation of several additional candidate genes has been studied in relation to prenatal stressors. Methylation status of *HSD11B2* (involved in glucocorticoid responses) and *SLC6A4*, but not BDNF, in neonatal cord blood were associated with prenatal socioeconomic deprivation (Appleton et al. 2013). In a well-powered study involving 500 pregnant women, the differentially methylated regions of several candidate imprinted genes were investigated and found to associate with severe maternal depression, increased low birth weight (Shapero et al. 2014), and heightened DNA methylation levels (Liu et al. 2012b). Finally, periconceptual exposure to famine during the Dutch Hunger Winter was found to associate with differential methylation of developmental and immunological genes in late adulthood (Tobi et al. 2009).

Several studies have tackled associations between prenatal stress and infant methylation at the genome-wide level. In one study, differential DNA methylation at *CYP2E1* (initially discovered via array findings and followed up by pyrosequencing the gene for greater coverage), a gene involved in metabolism, was predicted by maternal mood, selective serotonin reuptake inhibitor (SSRI) exposure, and their interaction. In turn, methylation status related to infant birth weight (Gurnot et al. 2015). In a prospective study, women were recruited who had experienced the 1998 Quebec Ice Storm while pregnant. Reported objective hardships experienced due to the disaster and subjective distress were related to methylation at numerous sites, with sets of CpGs reported that were both specific to and overlapping between objective and subjective measures. The functions of identified genes were predominantly related to immune function, and DNA methylation was found to mediate a

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link found between subjective stress and child immune and metabolic status (Cao-Lei et al. 2014).

In another study, minimal differences were found in DNA methylation in cord blood between infants born to mothers who had or had not experienced depression, with very weak differences reported for two significant CpG sites; however, the control group may not have sufficiently differed from the depressed group, as women in the control group had previously been diagnosed with mood disorders (Frey et al. 1990). In another study, nonmedicated depression or anxiety during pregnancy related to differential methylation at 42 CpG sites, with significant clusters related to the regulation of transcription, translation, and cell division. No differences were found between groups exposed to SSRIs *in utero* relative to controls (Non et al. 2014). A recent study compared the methylation profiles within buccal epithelial cells of infants with fetal alcohol spectrum disorder (FASD) and healthy controls, reporting 658 differentially methylated sites (Portales-Casamar et al. 2016). The majority of differentially methylated genes, when tested in cortical tissue from the Allen Brain Atlas, demonstrated high mRNA expression as well as high correlations with methylation patterns in corresponding buccal cells, supporting the potential functional significance of the sites identified in the FASD sample. Finally, one study assessed the link between prenatal environment and neonatal methylation, focusing on variably methylated regions, and tested whether several prenatal factors, genotype, or their interaction best explained methylation outcomes in independent models. Interestingly, the majority of variably methylated regions were best explained by an interaction between genotype and prenatal environment (Teh et al. 2014). It is possible that future investigations could more effectively detect prenatal environmental effects on DNA methylation if genotype is taken into account, as Teh and colleagues found that prenatal environment on its own was not the best predictor of DNA methylation in variable regions in any tested models.

Postnatal Environment

Similar to research on prenatal exposures, postnatal environments have demonstrated associations with methylation patterns in offspring at later developmental time points. The majority of these studies have targeted the candidate gene *NR3C1* (which raises the same concerns as mentioned for the prenatal literature above). In a recent systematic review of this candidate literature, it was reported that child adversity and parental stress in early life related to increased methylation (Turecki et al. 2016). One very small cohort study reported that childhood abuse was related to methylation of the GR promoter within hippocampal tissue of suicide victims, consistent with patterns reported for methylation in blood (McGowan et al. 2009).

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In another study the entire region of the *NR3C1* gene was investigated in the hippocampi of suicide victims who had or had not experienced abuse as children and compared to profiles obtained from rats. Numerous DNA methylation differences were identified that were conserved between human and mouse, and appeared to target regulatory sites such as gene promoters (Suderman et al. 2012).

Another popular candidate of interest is the serotonin transporter gene (5-HTT). Increased methylation of this gene was observed among monozygotic twins who were bullied relative to nonbullied siblings (Ouellet-Morin et al. 2013). In another study, a number of childhood adversities were significantly associated with 5-HTT promoter methylation status, as well as increased depressive symptoms (Kang et al. 2013). Finally, 5-HTT methylation has been linked to childhood abuse and sexual abuse (Beach et al. 2011).

Growing numbers of whole epigenome studies have tested associations between critical postnatal environments and DNA methylation patterns. In one study, maternal stressors during infancy and paternal stressors during preschool years were related to DNA methylation patterns in adolescents (Essex et al. 2013). Two studies have reported associations between methylation and early-life socioeconomic status: one suggested that a cluster of variably methylated CpG sites was correlated with socioeconomic status (Borghol et al. 2012); the other found socioeconomic status to associate with DNA methylation, perceived stress, cortisol, and inflammatory responses within peripheral blood mononuclear cells (Lam et al. 2012). These studies suggest that a broad and complex prenatal exposure, like socioeconomic status, may have implications for subsequent methylation profiles.

Several studies have investigated the effects of severe postnatal stressors on DNA methylation. In an investigation of methylation from peripheral blood mononuclear cells of adopted and nonadopted youth, adopted youth demonstrated substantial differences in white blood cell-type composition, as well as differences in methylation of genes functionally enriched for neural and developmental processes. Moreover, differences in methylation were only observed in relation to early and not later experiences of trauma (Esposito et al. 2016). In another study, orphanage rearing related to genome-wide increases in DNA methylation in the blood of children 7–10 years of age relative to parent-reared controls (Naumova et al. 2012). Finally, a study assessing differential methylation of gene promoters reported that DNA methylation differences in individuals who were abused as children were related to cell signaling pathways relevant to transcription regulation and development, including 39 miRNAs (Suderman et al. 2014).

In all, prenatal and postnatal environments appear to link to methylation signatures in neonates and across developmental time. The next body of evidence to be reviewed, linking epigenetic marks to neural outcomes, suggests that these environmental effects may indeed have implications for neurobiological development.

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Human Research on Link between Epigenetic Markers and Neural Outcomes

A nascent area of research has begun to target the structural and functional neural correlates of epigenetic patterns in humans. Given the empirically supported possibility that prenatal and postnatal environments modify epigenetic patterns, and that these critical environmental factors are linked longitudinally to physical and mental health outcomes, it is predicted that experience-dependent epigenetic marks induce functional consequences on developing neurobiology, or at least serve as biomarkers of experience-dependent neural development. The vast majority of this work has focused on DNA methylation, in both peripheral and central tissues.

Human studies relating methylation to brain function are largely limited to adult samples and single genes, but they suggest that the methylation of candidate genes relate to structure and function in regions responsible for emotion and stress regulation, such as the amygdala, hippocampus and prefrontal cortex (for a review, see Nikolova and Hariri 2015). (These candidate studies, however, suffer the same pitfalls mentioned above, in terms of focus on invariable promoter regions and failing to account for cell-type composition of samples.) Interestingly, some links between candidate gene methylation and functional activity have been further substantiated by molecular brain measures using positron emission tomography (PET). For instance, methylation of the 5-HTT promoter has been linked to limbic functional activity in response to emotional tasks in three studies (Nikolova et al. 2014; Frodl et al. 2015; Swartz et al. 2016). These functional MRI findings are consistent with a PET study which demonstrates that 5-HTT methylation status predicts serotonin synthesis within the orbitofrontal cortex, a critical region for higher-level emotional processing (Wang et al. 2012).

A recent study investigated the expression of hundreds of miRNAs following a social stress task in conjunction with functional imaging (Vaisvaser et al. 2016). The authors reported that the miRNA miR-29c expression in blood was related to perceived stress following the task, and differences in ventromedial PFC functional connectivity within regulatory regions. Vaisvaser and colleagues suggest that miR-29c may serve as a blood biomarker for neural responsiveness to stress.

To date, only three studies correlating DNA methylation to neural outcomes have additionally incorporated genotype. One well-executed study demonstrated that the association between childhood exposure to abuse and DNA methylation (from blood samples drawn during adulthood) of intron 7 of *FKBP5*, a functional regulator of the glucocorticoid receptor complex, was related to hippocampal volume and dependent on *FKBP5* genotype (Klengel et al. 2012). In a second study, genotype (Val66Met) and methylation of the BDNF gene, known to underlie synaptic plasticity was investigated (Chen et al. 2015). Chen and colleagues found that the degree to which antenatal

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maternal anxiety was associated with neonatal DNA methylation depended on BDNF genotype. Specifically, greater effects were observed for met/met genotype relative to met/val or val/val genotypes. A greater number of CpG sites were identified in which methylation level was related to right amygdala volume for met/met genotype; whereas the opposite pattern was observed for the left hippocampus, in which more CpG sites were correlated with volume for infants with the val/val genotype. This was the first study to demonstrate that an interaction between antenatal environment and genotype on the epigenome is reflected in substructures of the brain that are important for stress and regulation.

Finally, consistent with the notion that DNA methylation signatures may have implications for neurological function, studies on psychiatric disorders have reported links to epigenetic markers in peripheral and brain tissue. A few studies have compared methylation profiles in the peripheral tissues of monozygotic twins discordant for psychiatric disorders and found differences in the methylation of candidate genes related to neurotransmitter function (Petronis et al. 2003; Mill et al. 2006). In a genome-wide study, monozygotic twins diagnosed with major depressive disorder were reported to have greater variance overall in methylation relative to unaffected siblings (Byrne et al. 2013). A few human studies of major depressive disorder have validated epigenetic findings discovered in animal models of chronic stress (for a review, see Nestler et al. 2016). For example, a repressive histone mark, H3K27me3, implicated in the link between chronic stress and suppression of BDNF expression in the hippocampus (Tsankova et al. 2006) was found at elevated levels within the synapsin gene family of PFC tissue in individuals with depression and bipolar disorder (Cruceanu et al. 2013). An epigenome wide study of CpG-rich regions in PFC tissues of individuals with schizophrenia, bipolar disorder, and controls implicated a number of epigenetic modifications in these disorders related to glutamatergic and GABAergic signaling, neuronal development, and metabolism (Mill et al. 2008). Finally, at least two loci have been implicated in psychiatric disorders in multiple cohorts and across tissues: *HLA9* has been implicated in psychiatric disorders in postmortem brain tissue, blood, and sperm (Nestler et al. 2016) and *GAD1*, which originally demonstrated dysregulated expression and epigenetic differences in multiple brain tissues in schizophrenic individuals (Akbarian and Huang 2006), similarly demonstrated differential methylation in hippocampal tissue from individuals with bipolar disorder (Kaminsky et al. 2012). These studies provide promising evidence that epigenetic patterns found across tissues are relevant to the manifestation of psychiatric disorders.

A recent article highlights a promising possibility; namely, that epigenetic mechanisms may explain associations between genetic variability and psychiatric conditions (Bavamian et al. 2015). Specifically, the miRNA miR-34a, shown to be reduced by pharmacological treatment for bipolar disorder, was found to regulate two bipolar disorder risk genes during neuronal differentiation. Elevation of the expression of miR-34a affected mRNA and protein

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expression of these two genes and resulted in defects in neuronal differentiation, whereas suppression of miR-34a enhanced dendritic growth. Moreover, 25 genes found to be targeted by miR-34a overlapped with genes containing single nucleotide polymorphisms associated with bipolar disorder in genome-wide association studies. Thus, epigenetic mechanisms may ultimately help to elucidate the functional links between genetic variation and psychiatric conditions (Bavamian et al. 2015).

Synthesis of Human Research

Collectively, a growing body of human research has linked early environments to epigenetic patterns, and epigenetic patterns to neural and mental health outcomes. The following limitations associated with this literature must be emphasized (Jones et al. 2018). First, human research primarily relies on peripheral tissues, and methylation patterns may not be consistent between peripheral and central tissues. It is critical for studies that report DNA methylation findings, interpreted as potentially relevant to the brain, to utilize biobanks (e.g., Allen Brain Atlas) with peripheral and central tissues from the same individuals, and to report the correlations of methylation between tissues at discovered sites. Second, studies focused on candidate gene methylation should strategically select sites which are more likely to vary across individuals. As DNA methylation research continues to progress, it has become clear that methylation at gene promoters may not be as telling for individual differences or early exposures as originally anticipated, and that more variable DNA methylation sites with functional relevance may be found elsewhere (e.g., at enhancers in intergenic region). Third, a good deal of the early DNA methylation literature did not control for cell-type proportions in samples. Moving forward, it is highly recommended that studies utilizing array data use deconvolution methods to estimate proportions of cell types, and to control for these proportions or to sort cell types for the analysis of DNA methylation. Finally, a large proportion of the variability in DNA methylation is due to genetic influences (Henikoff and Greally 2016). Given the findings reviewed above (i.e., Teh et al. 2014), studies concerned with environmental effects on DNA methylation will be better positioned to detect these effects with accompanying genotype data so that those exposures with genotype-dependent effects can be identified.

Despite these limitations, consistent findings that DNA methylation correlates with early social environment and neural phenotypes (especially in studies that were well designed to account for the above issues) suggest that epigenetic marks capture meaningful variation in early environments as well as concurrent neurological measures and mental conditions. This evidence offers an intriguing possibility: that epigenetic marks in human central and peripheral tissues reflect an important biological substrate of experience-dependent

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plasticity relevant to current mental health status. As more studies begin to incorporate full genome and epigenome markers alongside neural measures, there will certainly be much to learn about the potential for epigenetic marks as (a) biomarkers of the developmental history of gene–environment interplay in the developing brain and (b) biological signatures relevant to functional neurobiology and associated mental conditions.

Conclusions

Epigenetic studies in humans offer an exciting new avenue for understanding how critical environmental factors induce enduring effects on the brain and behavior in human development. In addition to potentially guiding the expression of the genome in response to the intrinsic signals inherent to the processes of embryogenesis, neurogenesis, cell migration, synaptic transmission, and the timing of developmental windows, epigenetic regulators are also implicated in the embedding of early social experiences within neurobiology in an enduring fashion. These early modifications to the epigenetic code are hypothesized to have consequences for developing neural structures and function, channeling genetically driven effects on emotional and cognitive processes in a manner consistent with the emotional and social realities of an individual's outer experience.

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