Review

Epigenetics, brain, behavior, and the environment

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ABSTRACT

Early experiences can modify regulatory factors affecting gene expression in such a way that, although the DNA sequence itself is not changed, the individual's physiology and behavior is substantially influenced. In some instances these epigenetic effects are exerted upon exposure, while in other instances they are transmitted across generations via incorporation into the germline. Examples of both types of epigenetic effects are presented. First, experience with siblings (littermates) organizes behaviors and their underlying neural substrates in such a way that, as adults, rats and knockout mice behave differently. Second, exposure to the fungicide vinclozolin early in pregnancy imprints the male lineage in such a manner that rats exhibit distinct behavioral profiles as well as unique patterns of gene expression in relevant brain regions. Taken together, this work demonstrates that present and past environments alike modify both social and affiliative related behaviors and their related metabolic activity in specific brain nuclei as well as influencing the abundance of specific genes altering the epigenome in the target brain areas.

Key words: Behavior, Environmental influences, Epigenetics

INTRODUCTION

Many psychiatric disorders exhibit significant gender differences in relative risk level and severity. In women, the incidence of some disorders (e.g. eating disorders, major depressive disorder, obsessive-compulsive disorder, posttraumatic stress disorders, anxiety and panic disorders, seasonal affective disorder, and Alzheimer's disease and dementia) is two-fold

higher or more than in men. Males are at higher risk for early onset disorders such as autism and schizophrenia. It is clear that in some manner reproductive and adrenal hormones play a role in the development and display of these disorders since, in many instances, the sex differences manifest at puberty. In some cases the relationship is clear-cut, as is the case with stress hormones and anxiety-related behaviors. However, a direct causative (vs. consequential) nature of sex steroid hormones has been more difficult to demonstrate even though, for example, in the case of schizophrenia in women, worsening of symptoms is experienced during pregnancy, postpartum, and perimenopause.¹⁻³ A clearer picture emerges when we consider individuals exposed to exogenous steroids or their mimics. It is well known that conditions such as congenital adrenal

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hyperplasia, resulting in elevated androgen levels, influence subsequent gender identity and role.^{4,5} Even modestly higher *in utero* androgen exposure during fetal life has detectable effects in later adulthood. For example, a female dizygotic twin with a twin brother, as compared with a female dizygotic twin with a twin sister, exhibits more risk-taking behavior, has a more masculine pattern of cerebral lateralization, and is prone to aggression.⁶⁻⁸ Both men and women exposed to diethylstilbestrol (DES) *in utero* (hence having a body burden of the chemical) are more prone to depression compared to their unexposed siblings.⁹⁻¹¹ Indeed, an issue of national concern is the significant environmental exposure to common-use chemicals in the household, a factor suggested as contributing to the increased incidence of affective disorders in the general population.¹²

When and how are these gender biases established? Hormones and genotype determine an individual's responses to experiences throughout the life cycle as well as the susceptibility to developing disorders.¹³⁻¹⁵ Embryonic development is the time of maximal neuronal and behavioral plasticity, although the individual's capacity to respond to environmental change or insult with heritable phenotypic variation at a later stage is also possible. For the purposes of this essay, plasticity is defined as the ability of the genotype to produce different phenotypes in response to different environments. In both instances, suites of genes underlie the fundamental plasticity of an organism, particularly during development or life history transitions. Exploration of such gene-environment interactions furthers our understanding of how the environment influences the relationship between genotype and behavior during sensitive developmental periods.

Affective disorders result from the interplay of environmental, genetic, and epigenetic factors during neural development, but exactly how this comes about is relatively unknown.^{16,17} Recent studies at both the molecular and organismal levels indicate that the origin of such effects may be in previous generations. That is, experiences of earlier generations modify regulatory factors affecting gene expression in such a way that the DNA sequence itself is not changed but the individual's physiology and behavior are substantially influenced. Of particular interest is how such

effects might be transmitted across generations. First, it is important to distinguish mitotic (non-germline) from meiotic (germline) epigenetic imprints.

CONTEXT-DEPENDENT VS. GERMLINE-DEPENDENT EPIGENETIC MODIFICATIONS

Transgenerational effects can be observed if the environmental factors that bring about the epigenetic modification simply continue to persist.^{18,19} For example, if the diet, behavior (see below), or environmental toxicant (e.g. lead) continues to be present in the environment, then epigenetic modification will be manifested in each generation.^{20,21} This situation leads to readily available therapeutic venues such as those providing methyl donors to the diet or directly to the young, or simply removing the environmental toxicant.²²⁻²⁴ Hence, the environment can induce epialleles, but this environmentally induced epigenetic state can be reversed by a different environmental factor. I term this mitotically based transgenerational effect 'Context-Dependent' epigenetic change and the best example comes from the work of Meaney and colleagues.^{18,23,25,26} In a long series of elegant studies, this group has demonstrated that nature and the amount of care a pup receives from the mother modulates its reaction to stress later in life largely through effects on the glucocorticoid receptor (GR) in the hippocampus. This maternal effect can cross generations, provided that the pup's experience occurs in the first week of life. Recently, this group has documented that infusion of methionine, a histone deacetylase inhibitor, into the hippocampus can also reverse these events. Is there a counterpart in humans? Caspi and colleagues have demonstrated how the rearing environment can overcome the influence of genotype in the etiology of violent behavior.^{27,28}

Germline-Dependent epigenetic modification is fundamentally different than Context-Dependent epigenetic modification.¹⁸ This type of transgenerational epigenetic imprint is mediated through the germline and tends to be sex-linked. That is, an epigenetic modification is transferred to subsequent generations because the change in the epigenome is incorporated into the germline. Thus, the effect is manifested in each generation in the absence of the causative agent. In such instances the DNA methylation of heritable

epialleles is passed through to subsequent generations rather than being erased as occurs normally during gametogenesis and shortly after fertilization. It should be emphasized that Germline-Dependent epigenetic modifications are not equivalent to genomic imprinting in which genes are monoallelically expressed in a parent-of-origin dependent manner.^{29,30} In the latter case of genomic imprinting, subsets of genes are silenced and influence development; silencing of genes is erased and not transmitted to the next generation. To date, there is but a single example of Germline-Dependent epigenetic modification on behavior.

EPIGENETICS AND MENTAL HEALTH

A growing body of information suggests that epigenetic effects might extend to gender differences in brain and behavior.^{18,31} For example, Woolf and Grossman and colleagues have presented, compelling arguments that for certain psychopathological conditions (schizophrenia, fragile X syndrome, fetal alcohol syndrome, and depression), early developmental events canalize the individual into an ever-narrowing range of responses.^{32,33} It is significant that methylation has been implicated in the etiology of all of these disorders.

More than 70 years of research with animal models has demonstrated that gonadal and adrenal hormones organize the brain perinatally in such a way that the individual's perception, behavior, and learning abilities are modified. Not only does the nature and amount of hormone affect individual development but also the timing of hormone exposure is important. However, while the principle of critical periods of hormone sensitivity is well established in animal studies, the data on human behavior is only now being collected.³⁴ Perhaps the best studied phenomenon is that of stress and how, if it is sustained, it can lead to impaired immunity, disease, and neurological changes characteristic of major depressive illness and particularly chronic anxiety disorders.³⁵⁻³⁷ Chronic restraint stress in rats has been a standard paradigm for studying such effects on physiology, brain, and behavior. For example, six hours daily of immobilization restraint for three weeks results initially in elevated corticosterone levels but after 21 days, the hypothalamus-pituitary-adrenal (HPA) axis shows ad-

aptation and levels are back to normal. However, there is a progressive atrophy of the dendrite length and branching of pyramidal neurons in the CA3 region, a process mediated by corticosterone potentiating the release and postsynaptic activity of excitatory amino acids from adjacent mossy fiber terminals arising from the granule neurons in the dentate gyrus and acting via N-Methyl-D-Aspartate (NMDA) receptors.³⁸ Conversely, there is an increase in dendritic spine density of neurons in the basolateral amygdala and Medial Prefrontal Cortex (mPFC), and decreased neurogenesis in the dentate gyrus.³⁹⁻⁴³ In addition to suppressing proliferation of new cells, chronic restraint markedly increases polysialic acid, a developmentally regulated carbohydrate associated with Neural Cell Adhesion Molecule (NCAM).⁴⁴ Stressed rats also exhibit a variety of specific cognitive deficits in spatial learning and memory as well as increased anxiety-like and agonistic behavior.^{39,45,46}

The effects of stress appear to vary depending upon the sex of the individual and when they occur.⁴⁷ For the purposes of this essay, I will only consider the literature on males. In males, the effects of chronic stress early in development tend to be irreversible, resulting in permanent structural changes in the hippocampus and altered adult sociosexual and anxiety-related behaviors, while those experienced as an adult can be reversed. If the stress occurs during the peripubertal-juvenile transition, the effects are similar to the early effects, if not exaggerated.⁴⁸ In rats, chronic restraint influences serotonin and dopamine activity in CA3 of the hippocampus, dopamine and its metabolites in CA1 of the hippocampus as well as the mPFC, and dopamine and its metabolites in the basolateral amygdala.

The context in which the stress is experienced is also important, perhaps not surprisingly as rats and humans are social animals. For example, stress decreases neurogenesis in the dentate gyrus of male rats that are individually housed but not in those that are socially housed.⁴⁹ In humans, some disorders are precipitated by stress, which itself alters endocrine state. For example, only some of the pregnant women exposed directly to the World Trade Center collapse developed Posttraumatic Stress Disorder (PTSD).⁵⁰ These women and their babies had lower cortisol levels compared with those mothers who did not develop

PTSD. The issue here is whether stress potentiates a predisposition to develop mental disorder(s). A similar point has been raised by Petronis and colleagues regarding pre- and perinatal environmental risks for Attention-Deficit Hyperactivity Disorder (ADHD).^{5,51}

It is now well accepted that life history events interact with genetic predispositions to induce disease. For example, Eker rats carry a germ line defect in the tuberous sclerosis complex 2 tumor-suppressor gene and approximately 65% develop hormone-dependent uterine leiomyomas.⁵² Exposure of females to DES on days 3-5 after birth increases the tumor-suppressor-gene penetrance to more than 90%. In principle, stress, which has been shown to have organizational actions of behavior in rats via an effect on the HPA, could have a similar potentiating effect on epigenetically induced transgenerational imprints as well as on genetic predisposition to develop disease. Indeed, glucocorticoid receptor regulates DNA methylation within a key enhancer of the rat liver-specific tyrosine aminotransferase gene, resulting in rapid chromatin remodeling. This demethylated state is stable and results in an enhanced hormonal response to glucocorticoids on additional exposure.⁵³ An intriguing recent finding concerns differences in D2 Dopamine Receptor (DRD2) variants in PTSD patients that are co-morbid for anxiety, social dysfunction, and depression; methylation plays a role in DRD2 expression.⁵⁴⁻⁵⁸

Finally, perhaps the best evidence for epigenetic influences in affective disorders comes from studies of Monozygotic (MZ) twins.^{2,59} In such instances, the concordance rate for both of the twins to suffer from an affective disorder is higher than that observed in dizygotic twins or sib pairs (e.g. schizophrenia-70% and autism-60%). The fact that it is not complete is of interest but outside the scope of this brief review. Epigenetic MZ twin differences have been identified that vary with age, but also between twin pairs.^{60,61} For example, the Catechol-O-Methyltransferase (COMT) gene is located on chromosome 22q11, a region implicated in the etiology of schizophrenia.⁶² Mill et al. studied the concordance rate for CpG methylation in the promoter region of this gene in 12 5-year-old MZ twin pairs discordant for birth weight, finding a range of concordance from <1 to 42%.⁶³

More relevant to this discussion, a comparison of two MZ twin pairs, one concordant for the diagnosis of schizophrenia and the other discordant, revealed larger epigenetic difference in the regulatory region of the DRD2 gene.⁵⁶

NEURAL MECHANISMS UNDERLYING SOCIOSEXUAL AND EMOTIONAL BEHAVIORS

There is now a substantial body of literature indicating that in mammals, specific nuclei in the limbic and forebrain areas are critical to the display of reproductive, agonistic, and emotional behaviors.⁶⁴⁻⁷¹ These nuclei, which include the lateral septum, amygdala, hippocampus, bed nucleus of the stria terminalis, medial preoptic area, anterior hypothalamus, and ventral tegmental area, are interconnected, contain steroid hormone receptors, and tend to be sexually dimorphic in their volume and synaptic organization as a consequence of the nature and frequency of sex steroid hormones secreted perinatally. Ablation of individual nuclei often leads to diminution or even abolition of social and reproductive behaviors, whereas stimulation in individuals gonadectomized in adulthood restores these behaviors. Stress influences cognition and anxiety, effects that are sexually dimorphic and hormonally modulated. Moreover, the functional neuroendocrinology of brain areas associated with stress have been delineated (see above and below). The display of these complex behaviors is reflected in increased expression of immediate early genes as well as electrophysiological and metabolic activity in multiple nuclei that can form an integrated neuronal circuit. This has led to increased appreciation that social and reproductive behaviors “emerge from the activity of a unitary neuroanatomical framework” in the brain.⁷² The Crews lab has been at the forefront of developing analytic methods for evaluating change in such networks.⁷³

CONTEXT-DEPENDENT EPIGENETIC CHANGES IN BRAIN AND BEHAVIOR

Life history is continuous but can be viewed as the cumulation of discrete segments; each period emerges from what goes before and, at the same time, sets the stage for what follows. Although the divisions are somewhat arbitrary and some traits can

span conventional divisions, in mammals the usual classification is prenatal (intrauterine), postnatal (until weaning), adolescence (after weaning), peripubertal, sexual maturity, and reproductive senescence. Each period has its own characteristic ethologies and particular contribution to the behavioral phenotype. It is possible to deconstruct early life events and study each period both in its own right and how it interacts with the other stages.

In the field of behavioral neuroscience, complex behavioral traits are typically studied in the adult organism. In mammals, the formative environment for social and anxiety-related behaviors is the family unit; in the case of rodents, this is the litter and the mother-young bond.^{25,74,75} Normally, investigators utilize individuals without consideration of the litter in which they were born. However, the litter is a structured unit involving the mother and her life history as well as the pups as they interact with one another and with the mother and research has demonstrated how much of adult behavior has its antecedents early in life.

A deciding factor in this environment is the sex ratio of the litter and, in the case of mice lacking functional copies of gene(s), the ratio of the various genotypes in the litter. Recent studies have deconstructed these two confounds and demonstrated that they have separate and distinct effects on the nature and quality of the individual's behavior later in adulthood, as well as on the metabolic activity in brain nuclei related to these behaviors.^{73,76,77} The finding that functional neural systems can be re-organized, depending upon the composition of the litter in which the individual develops, is startling. Yet it yields a deeper understanding of how neural systems are organized early in life.

Normally, litter composition reflects the sex ratio produced at birth, but there is evidence that prenatal environment (who your fetal neighbors are) and the postnatal period (the nature and quantity of maternal care) affect the adult behavioral phenotype. However, in none of these studies have these two periods been disassociated. Specifically, research demonstrating that the intrauterine sex ratio influences adult behavior failed to control for sex ratio of the litter postnatally. Similarly, research demonstrating that

the sex ratio of the litter influences maternal behavior has not taken into account the prenatal sex ratio of the pregnant mother.

However, and contrary to the literature, deconstructing these sequential experiences reveals that it is the sex ratio of the litter postnatally that affects sexuality in adult males, not intrauterine position or maternal behavior.^{73,78} After controlling for prenatal sex ratio, we find that males raised in female-biased litters exhibit less mounting compared to males raised in litters of equal sex ratio or in male-biased litters. Further, males from female-biased litters are less attractive to sexually receptive females. These differences are not erased by sexual experience, suggesting that the effects of the sibling environment are permanent. Surprisingly, these males compensate for their lower attractiveness by being more efficient copulators.

In genetically-modified mice, not only is the sex ratio of the litter an issue but the ratio of the various genotypes is an equally important variable, particularly in model systems that are the result of the mating of Heterozygotes (HTZ) to yield litters of varying numbers of Wildtype (WT), HTZ, and Knockout (KO) young of both sexes. Typically, researchers using KO mice do not control for the early social environment of their experimental animals. This is a mistake since this early social environment has a powerful effect on shaping the adult behavioral phenotype and brain. A common mouse model is the Estrogen Receptor α (ER α) knockout that lacks a functional copy of this important sex steroid receptor. Not only has research revealed the role of this gene in differentiation of morphology and physiology but distinct behavioral phenotypes have also been characterized. The question is, to what extent are the behavioral phenotypes due to the absence of the gene or to the litter in which the individual develops.

Not only is it possible to distinguish males and females on the day of birth but it is also possible to genotype each individual in the litter using PCR to distinguish WT, HTZ, and KO individuals. Using this approach litters were reconstituted to control for sex ratio and genotype ratio.^{76,77} Results indicate that sex and genotype of siblings in the litter affected aggressive behaviors as well as patterns of metabolic

activity in limbic nuclei in the social behavior network later in adulthood. Moreover, this pattern in males varied depending upon the genotype of their brothers and sisters. Principal components analysis revealed two components comprised of several amygdala and hypothalamic nuclei; the Ventromedial Hypothalamic nucleus (VMH) showed strong correlations in both clusters, suggesting its pivotal nature in the organization of the two neural networks. For example, WT females spend significantly more time in social contact in a Resident-Intruder test compared to KO females raised in same-sex, same-genotype litters. Further, it appears that female WT siblings are able to compensate for this deficit, just as KO siblings cause a deficit in WT females.

Cytochrome Oxidase (CO) histochemistry is a particularly useful tool for studies of the long-term effects of significant life history events. The abundance and activity of CO in a brain area is a measure of the metabolic capacity of that brain region. In other words, the CO abundance not only reflects the metabolic history of an area but, because it determines the amount of ATP available in a neuron, constrains the amount of activity a neuron can sustain.⁷⁹ Using this tool, we find that the neural networks that subserve sociosexual behavior vary in different ways. First, there is a significant genotype difference in the neural network of WT and KO mice and the compensation/deficit in the behavior are reflected in the metabolic activity of the neural circuit. The relative effects of sex, independent of genotype, and of genotype, independent of sex, are striking. Taken together, these findings indicate that in studies with genetically modified mice, the litter composition during the pre-weaning period must be considered as it can effect the development of behavior and the neural network responsible for the regulation of emotional behaviors.

TRANSGENERATIONAL EPIGENETIC PROGRAMMING OF THE BRAIN TRANSCRIPTOME AND ANXIETY BEHAVIOR

Michael Skinner and colleagues have developed a rat model in which the male germline bears a permanent epigenetic imprint, thereby creating an epigenetic transgenerational phenotype that is not

Context-dependent.⁸⁰⁻⁸³ This demonstrates that exposing gestating female rats to Vinclozolin during the period of sex determination induces an epigenetic transgenerational phenotype through reprogramming the germline in a sex-specific manner. Specifically, in each generation males whose ancestor had been treated showed accelerated onset of adult diseases such as cancer, prostate disease, kidney disease, and immune defects. The appearance of a series of new imprinted-like genes that transgenerationally transmits this altered epigenome to promote disease phenotypes appear not only in the sperm epigenome but also in the brain epigenome.^{82,83} Recently, a Germline-Dependent epigenetic modification effect on mate preference has been demonstrated.⁸⁴

The transcriptomes of the whole brain, amygdala, and hippocampus of these same F3 generation Vinclozolin-lineage and Control-lineage males show the same trends in expression with the COMT microarray results, demonstrating a decrease in all the vinclozolin gene sets in both the amygdala and hippocampus, but to a lesser extent in the latter.⁸⁵ Hundreds of genes have altered expression in a transgenerational manner. Of these, a limited number show similar changes in the whole brain, amygdala, and hippocampus. Genes common to all three include Senp5 (SUMO/sentrin specific protease 5), Nfix (Nuclear factor I/X), Akap5 [A kinase (PRKA) anchor protein 5], NTrkb (Neurotrophic Tyrosine kinase receptor) and COMT, the latter three genes having been implicated in the etiology of schizophrenia and other affective disorders including autism and depression.^{62,86-89} Camk2a (calcium/calmodulin-dependent protein kinase II alpha subunit) was also regulated in the amygdala and hippocampus, this being a gene implicated effect in both learning and memory and stress-induced anxiety behavior.^{90,91}

Vinclozolin-lineage males spent more time in the light compartment and had more transitions than did control males. In the Elevated Plus maze, there was no difference between the lineages in % open arm time and entries, but with significantly greater total arm entries for the Vinclozolin-lineage males.⁸⁵ Taken together, these studies indicate that the epigenetic transgenerational phenotype has a permanent alteration in the brain transcriptome in a manner that can

influence behavior and genes implicated in anxiety-related disorders, learning, and memory.

Surveying the recent literature for genes implicated in mental disorders reported by multiple laboratories and comparing it with our brain transcriptome analysis reveals that in the whole brain AUTS1 (candidate gene in region of chromosome 7 known as autism susceptibility locus 1) and Grik2 (Glutamate receptor, AMPA1) are decreased, while SLC6A4 (Serotonin transporter gene), Gria1 (Glutamate receptor, kainite 2), and S100 (calcium binding protein A4) are increased; the latter gene also shows a specific increase in the amygdala. Interestingly, AUTS2 (autism susceptibility locus 2 or engrailed homolog- EN2- in the rat) is not affected.^{24,92-95} (AUTS1 remains a strong candidate gene involved in autism, but AUTS2 is no longer considered to be so.⁹⁵) Thus, it is significant that the AUTS1 and AUTS2 candidate genes map to the chromosome 7q location, the same region as Candidate gene 23 described by Chang et al; further, AUTS1 is reduced by the Vinclozolin-lineage males, but not AUTS2.⁸³ The amygdala also showed an increase in expression in BDNF, DRD2, and S100. The increases in BDNF and DRD2 in amygdala are significant as the former has been implicated in Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence, while the latter has been implicated in PTSD, anxiety, social dysfunction, and depression.^{54,96}

It is potentially instructive that a number of genes in the same literature were not found to be different in the Vinclozolin imprinted rat. For example, the gene *RELN* is downregulated in the schizophrenic brain and it has been suggested that this is due to hypermethylation of the *RELN* promoter.^{97,98} There is no evidence that the Vinclozolin-lineage male brain is different from the control-lineage male brain in this regard. The stability of these genes in face of epigenetic imprinting will enable them to be used as benchmarks for the effects of stress. Finally, other genes that have been implicated in autism and related affective disorders such as *MECP2*, *GAD67*, and *IMMP2L* (= *IMP2*) are not on the Affymetrix chip RAT230-2.0 used and so could not be evaluated. At this point there does not appear to be a rat homolog for *NLGN4*.⁹⁹

CONCLUSIONS

The way the genetic background modifies responses to experiences throughout the life cycle can ultimately determine an individual's susceptibility to developing affective disorders. Recently, it has been discovered that early experiences can modify regulatory factors affecting gene expression in such a way that the DNA sequence itself is not changed but for generations afterwards an individual's physiology and behavior are substantially influenced. How this epigenetic modification can modulate the interaction of the environment and genetic constitution at the level of the brain, ultimately influencing agonistic and anxiety behaviors, is the next frontier.

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