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Development and the epigenome: the 'synapse' of gene–environment interplay

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Abstract

This paper argues that there is a revolution afoot in the developmental science of gene–environment interplay. We summarize, for an audience of developmental researchers and clinicians, how epigenetic processes – chromatin structural modifications that regulate gene expression without changing DNA sequences – may offer a strong, parsimonious account for the convergence of genetic and contextual variation in the genesis of adaptive and maladaptive development. Epigenetic processes may play a plausible explanatory role in understanding: divergent trajectories and sexual dimorphisms in brain development; statistical interactions between genes and environments; the biological embedding of early psychosocial adversities; the linkages of such adversities to disorders of mental health; the striking individual variation in the strength of those linkages; the molecular origins of critical and sensitive periods; and the transgenerational inheritance of risk and protection. Taken together, these arguments converge in a claim that epigenetic processes constitute a promising and illuminating point of connection – a 'synapse' – between genes and environments.

Introduction

At the October 1889 meeting of the German Anatomical Society in Berlin, celebrated Spanish neuroanatomist Santiago Ramón y Cajal presented his histological slides of brain tissue stained using the technique developed by his contemporary, Italian Nobel laureate Camillo Golgi. The slides revealed the microanatomic structure of the brain, consisting not of the gelatinous syncytium or the fibrous reticulum it had been previously assumed to be, but rather a breathtakingly complex network of individual cells, an immense sea of 'neurons'. In addition to the brain's cellular composition, however, Cajal's stained histological sections showed a vast assembly of 'synapses', clearly visible points of communication between neurons, numbering in the thousands per individual cell. This demonstration of the microanatomic, *physical nexus* between the billions of neurons in the human brain became the provenance of much of what we now recognize as 21st-century neuroscience: the understanding of molecular neurotransmission between neurons; the electrophysiology of circuitry formation and activation; and the use of psychopharmacological agents to alter functional relations among brain structures.

This paper will argue that the emerging science of epigenetics is now poised to similarly revolutionize knowledge of human development and its perturbations, by locating *physical points of connection* between genes and environments and by revealing a molecular embodiment of gene–environment interplay in its propagation of disordered or healthy development. By 'gene–environment (G–E) interplay', we refer to the broad co-action of genes and environments on developmental and health outcomes (Rutter, 2010). The term 'epigenetics', in its invocation of G–E interplay, was first used within a developmental context in a 1942 paper by biologist Conrad Waddington to reference a field of study exploring the processes by which genotypes are functionally linked to adult phenotypes. Waddington argued that cell differentiation involved an epigenetic 'canaliza-

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tion' of development, analogous to marbles rolling down a 'landscape' of deepening troughs or canals, seeking the surface's lowest points, within increasingly irreversible, or canalized, trajectories. What began as an intuitive metaphor describing crudely observable cell differentiation has become – in the years since 1942, and exponentially within the last two decades – a science occupying the burgeoning epicenter of a new developmental biology. As illustrated in Figure 1, concepts of epigenetic development, as well as an explicit, molecular science of epigenesis, have dramatically flourished in the years since Waddington's observation, with nearly 20,000 publications bearing the key words 'epigenetic', 'epigenesis' or 'epigenetics' and listed in PubMed over the first four years of the current decade.

Chromatin structure

More recently, epigenetics (from the Greek root *epi*, meaning upon or over) has been defined as 'the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered [gene] activity states' (Bird, 2007). Epigenetic mechanisms change gene activity or expression by altering chromatin organization, without modifying the genetic code of the DNA (Meaney, 2010). Such a definition carries with it the implication that the epigenome is a responsive overlay on the genome itself, possibly buffering or moderating genetic variation through up- and down-regulation of gene transcription. *Chromatin* is the physical packaging of DNA within chromosomes, in a structural configuration shown in Figure 2. Its most basic unit is the *nucleosome*, which comprises 147 base-pairs of DNA wrapped around a histone protein octamer.

The 30 million nucleosomes in each cell effectively reduce the length of DNA from 2 meters to a packageable 28 centimeters, thereby appearing in electron microscopy as a configuration resembling beads on a string (each 'bead' constituting a single nucleosome). Several additional, higher levels of chromatin organization also exist, ultimately sculpting the typical structure of chromosomes and condensing the length of DNA to fit into a cell nucleus. In broader chromosomal regions, often visible in microscopy stains as chromosomal bands, chromatin can be loosely or tightly structured (called euchromatin and heterochromatin, respectively, and also illustrated in Figure 2), thus offering either straightforward or more difficult physical access of the transcriptional enzyme, RNA polymerase II, to gene promoter and coding regions. Chromatin configuration is controlled physicochemically by the placement or removal of chemical tags – i.e. epigenetic 'marks', such as methyl, acetyl, phosphate, or ubiquitin groups – on the DNA or histone proteins (also shown in Figure 2).

DNA methylation, histone modification, and microRNA

The most highly studied and best characterized epigenetic mark, DNA methylation, involves a direct covalent, chemical modification of a cytosine base lying sequentially adjacent to a guanine base (thus a *CpG dinucleotide*); such methylation is a relatively stable epigenetic tag, catalyzed by a group of enzymes called DNA methyltransferases (DNMTs). CpG methylation can occur during any stage of the cellular life cycle and, depending upon genomic context, can impede or foster gene expression or leave it unchanged (Klengel, Pape, Binder & Mehta, 2014). CpG dinucleotides are relatively infrequent in the genome, and areas of comparatively high CpG content have been termed 'CpG islands' (Illingworth & Bird, 2009). CpG islands tend to be hypomethylated compared to other CpG sites and are found associated with approximately 70% of known gene promoters, i.e. the regulatory, non-coding portion of a gene that plays a role in transcription control (Saxonov, Berg & Brutlag, 2006; Illingworth & Bird 2009). Promoter DNA methylation (often in CpG islands) and gene body DNA methylation generally show

Figure 1 *Percentage of words 'epigenetic', 'epigenesis' or 'epigenetics' within Google Ngram English books corpus, 1800–2000 (https://books.google.com/ngrams/); and number of PubMed citations with same three search terms, 1960–2014.*

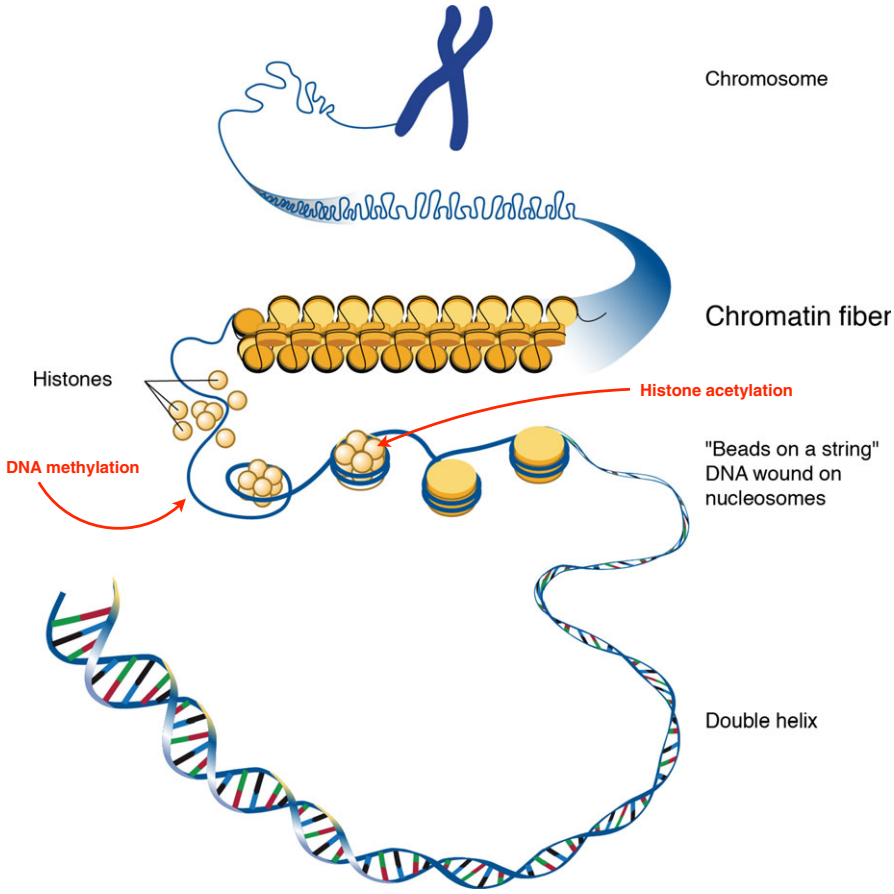

Figure 2 *The chromatin packaging of DNA wound around histone protein octamers, like 'beads on a string' (Darryl Leja, National Human Genome Research Institute).*

opposite associations with gene expression. High levels of promoter DNA methylation are frequently linked with diminished expression, but in the gene body, high methylation is more often coupled with augmented expression (Kass, Landsberger & Wolffe, 1997; Jones, 1999). Importantly, recent work in larger cohorts, on inter-individual variation, has shown that these principles hold only when looking across all genes within an individual. When comparing a single gene across individuals, the relation between methylation and expression could be negative, positive, or null (Lam, Emberly, Fraser, Neumann, Chen *et al.*, 2012; Gutierrez-Arcelus, Lappalainen, Montgomery, Buil, Ongen *et al.*, 2013). Such associations are therefore not straightforward, and concomitant gene expression is an important additional component of epigenetic analysis.

DNA methylation, with its accompanying down-regulation of gene expression, has only recently become viewed as reversible and thus a potential mechanism of developmental plasticity (Wu & Zhang, 2014) and a useful target of pharmacological interventions (Bojang & Ramos, 2014). Demethylation can occur actively or passively (Gibbs, van der Brug, Hernandez, Traynor, Nalls *et al.*, 2010; Bell, Pai, Pickrell, Gaffney, Pique-Regi *et al.*, 2011; Lam *et al.*, 2012), and active demethylation has been shown to occur via hydroxymethylation of CpG dinucleotides (Tahiliani, Koh, Shen, Pastor, Bandukwala *et al.*, 2009; Jones, Fejes & Kobor, 2013). The hydroxymethylation mark has also been hypothesized to have a functional role outside of demethylation, but as it is present in significant amounts only in neural and pluripotent cells, this function is not likely to be important in non-neural tissue (Lister, Pelizzola, Dowen, Hawkins, Hon *et al.*, 2009; Globisch, Muenzel, Mueller, Michalakis, Wagner *et al.*, 2010).

In a second form of epigenetic mark, post-translational modifications of the nucleosome's histone proteins are reversible chemical tags on the N- and C-terminal histone tails, with acetylation serving a permissive role in gene transcription through a relaxation of chromatin structure. Methylation of CpG sites, which occurs disproportionately in gene promoter

regions or near transcription start sites in the DNA sequence, results either in an interference with transcription factor binding or in the recruitment of methyl-DNA binding proteins, which in turn bring histone deacetylases (HDACs) to the site. The deacetylation of a histone protein causes, in turn, a compaction of chromatin structure and a consequent physical impediment to gene transcription. DNA methylation and histone deacetylation thus work hand-in-hand to allosterically down-regulate the transcriptional expression of a gene.

Because of the number, complexity and interdependence of histone modifications (14 distinct marks occurring in 100 sites on histone proteins), the concept of a 'histone code' or 'histone language' has been advanced, in which differing combinatorial patterns of modifications would drive certain transcriptional and epigenomic states (Strahl & Allis, 2000; Bridi & Abel, 2013). Such a pattern-language has been linked, moreover, to neuronal storage and the expression of memories and behaviors and may be associated, as well, with neuropsychiatric conditions such as cognitive impairments, schizophrenia and depression. Chronic stress down-regulates brain-derived neurotrophic factor (BDNF) expression, for example, through histone acetylation at the BDNF gene promoter region, an effect that is reversible with an antidepressant medication (Shirayama, Chen, Nakagawa, Russell & Duman, 2002). As a consequence of such observations, pharmaceutical HDACs have become a target of new drug discovery efforts in the treatment of cancer, neurological and psychiatric disorders.

A third, newly discovered epigenetic mechanism is the expression of small, non-coding RNAs (ncRNAs) that interfere with the expression of specific genes by associating with DNA to promote compacted heterochromatin formation or by fostering the degradation of transcribed messenger RNA (Mattick, 2010). Such ncRNA molecules are highly enriched in the mammalian brain. As with other epigenetic modifications, however, ncRNAs can serve multiple functions, including the activation or repression of gene expression, and have been linked to several disorders of cognition and behavior. For example, in Fragile X syndrome, a heritable disorder causing mental retardation and autistic-like behavior, a large, trinucleotide repeat sequence in the *FMR1* gene coding for Fragile X mental retardation protein (FMRP) results in its hypermethylation and transcriptional silencing. As a translation repressor, ncRNAs are thought to play an additional role in FMRP expression by targeting *FMR1* transcripts, impairing synaptic plasticity, and leading to mental retardation.

These three distinctive forms of epigenetic processes – each equipped to regulate and/or signal levels of gene expression – are not entirely independent, however, and recent research has shown how histone methylation can suppress nearby DNA methylation and how small ncRNAs and CpG methylation can reciprocally influence each other's presence and effects (Klengel *et al*., 2014).

Epigenetic differentiation of cells in embryogenesis

These processes of transcriptional control play an essential role, through the sequenced complexities of embryogenesis, in the ontogeny of a human body comprising ~200 different histological cell types, each containing the same genome, with the same DNA sequence. This proliferation of *cellular diversity from a genomic singularity* occurs during post-conceptual development by virtue of a highly regulated, epigenetic calibration in the expression of the ~20,000–25,000 protein-coding genes. Of the ~28 million CpG sites in the human genome (a smaller subset of the genome's ~one billion cytosine bases), as many as 60–80% are methylated within somatic cells (Wu & Zhang, 2014), and during mitosis, the pattern of DNA methylation is replicated in daughter cells, thereby maintaining cellular transcriptional memory. Some deviation from exact replication occurs for both epigenetic marks and DNA sequence, but the rate of stochastic errors in the former is estimated to exceed that of the latter by a factor of three (Petronis, 2010). Gene transcription, and a cell's histological fate, is thus controlled by epigenetic marks acquired as part of the cell differentiation process and are dependably reproduced, to a remarkable degree, during DNA replication and cell division.

Early embryologic processes also depend critically upon the epigenetic programming that underlies cell differentiation and development (Smith & Meissner, 2013). As depicted in Figure 3, methylation of DNA from primordial germ cells undergoes a global reversal, followed by an extensive, gamete-specific re-methylation process and complex epigenetic remodeling (Strachan & Read, 2011). Genomic imprinting, which occurs in nearly 400 human genes, involves epigenetic modifications of either DNA or histone proteins acquired from one parental gamete, resulting in only the other parental gene copy being expressed. After the two parental pronuclei – from egg and sperm – have fused, the early zygotic genome undergoes a second, expansive demethylation. Starting at the blastocyst stage of embryogenesis, there is another genome-wide, *de novo* methylation process that establishes and maintains distinctive cell

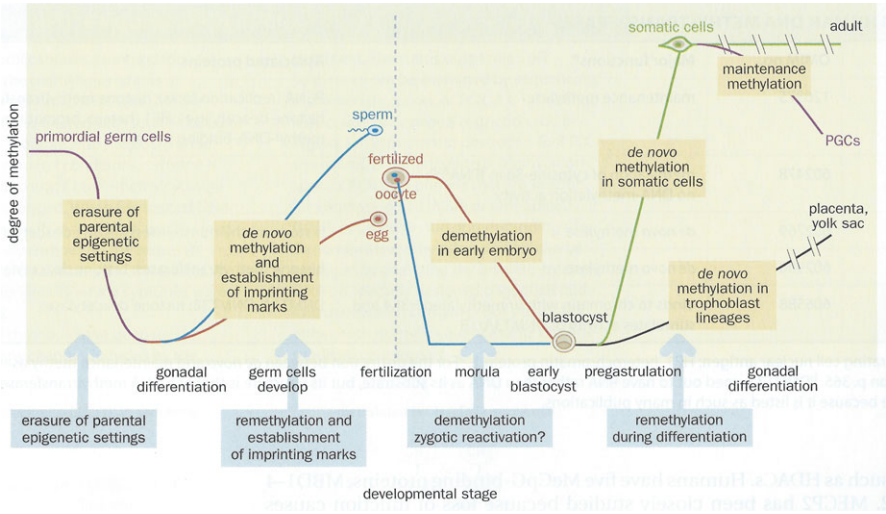

Figure 3 *DNA methylation/demethylation during embryogenesis (Strachan & Read, 2011).*

lineages, giving rise to the broad array of somatic cell types, as well as the trophoblastic cell lines that form the placenta and other reproductive structures. This phasic, genome-wide erasure and reconstitution of methylated cytosine nucleotides, at the dawn of embryogenesis, creates a kind of genomic *tabula rasa* upon which the epigenetic reprogramming of cellular and organismic diversity can be written. The epigenetic profile of a cell then becomes the mechanism whereby a liver cell 'remembers' that it is a liver cell over an entire human lifespan.

Neurodevelopment, dynamic variation and an epigenetic paradox

Interestingly, however, the very same processes that instantiate this ontogenetic *stability* in cell differentiation are those that also ensure *dynamic variation* in transcriptional activity in response to environmental signals and conditions. Cellular activity, such as the depolarization of neurons during neural circuitry activation, induces moment-to-moment shifts in gene activation or deactivation. In one example elucidated by Lubin *et al.* (Lubin, Roth & Sweatt, 2008), exposure to a fear learning procedure in rats produced an activity-dependent, dynamic regulation of the *BDNF* gene in the hippocampus – by DNA methylation, in the absence of cell division, and in response to environmental influences. Such epigenetic regulation is ubiquitous in the brain and is responsible for a variety of complex neural functions, such as memory formation, learning and the calibration of stress response circuitry (Bohacek, Gapp, Saab & Mansuy, 2013).

Widespread and regionalized shifts in DNA methylation and histone modifications have been shown to take place concurrently with key phases of normal brain development, such as synaptogenesis (Lister, Mukamel, Nery, Urich, Puddifoot *et al.*, 2013; Shulha, Cheung, Guo, Akbarian & Weng, 2013), and in association with specific disorders of development and mental health (Champagne, 2010; Toyokawa, Uddin, Koenen & Galea, 2012; Kofink, Boks, Timmers & Kas, 2013). Indeed, it appears that the entire process of mammalian neurodevelopment requires a precisely coordinated sequence of epigenetic events – involving genomic methylation and demethylation – in order to produce and spatially locate functionally distinct populations of neurons and glia cells (Wu & Zhang, 2014). Further, Qureshi and Mehler (Qureshi & Mehler, 2010) and Hodes (Hodes, 2013) have reviewed evidence that DNA methylation, histone modifications and ncRNAs may all be implicated in the known sexual dimorphisms arising during brain development, which may underlie differential susceptibility among males and females to various forms of psychopathology. Epigenetic links to neurodevelopmental abnormalities and disorders of mental health include the distinctive methylation patterns found within hundreds of gene loci, among patients with autism spectrum disorder and other neurodevelopmental syndromes (Shulha, Cheung, Whittle, Wang, Virgil *et al.*, 2012; Berko, Suzuki, Beren, Lemetre, Alaimo *et al.*, 2014). Children with Down Syndrome, with their triplication of chromosome 21, should theoretically have 50% more expression of those chromosomal genes, but their actual transcription varies from that expectation, suggesting that DNA methylation, histone modification and

possibly other epigenetic events may be involved (Dekker, De Deyn & Rots, 2014). Such differences in epigenetic 'adaptation' might give rise to the cognitive deficits that co-occur in Down Syndrome, rendering them potentially amenable to pharmacological interventions targeting the epigenome.

Thus, in an 'epigenetic paradox', the molecular sources of longitudinal cellular stability, by which histological differentiation occurs in early embryogenesis, are co-opted to subserve the organism's finely tuned, short- and long-term responsivity to shifting environmental circumstances. Such responsivity is neither fully environmental nor fully genetic in origin, but rather a synthesis, a convergence of gene and context. For a more complete treatment of the molecular and developmental processes summarized here, the reader is referred to books by Allis and colleagues (Allis, Jenuwein, Reinberg & Caparros, 2007), Gilbert and Epel (Gilbert & Epel, 2009), and Sweatt and colleagues (Sweatt, Meaney, Nestler & Akbarian, 2013).

Tissue specificity of epigenomic variation

Closely related to this paradoxical colocation of cellular stability and change within a single epigenomic structure is the dilemma of how to study dynamic epigenetic change against a backdrop of systematic, tissue-specific differences in epigenetic profiles. How can the epigenetic modifications associated with early adversity in hippocampal neurons – arguably a logical target tissue in which to search for such effects – be reliably discerned from those found in buccal epithelial cells (BECs)? What can chromatin modifications found in peripheral blood mononuclear cells (PBMCs) possibly reveal of the epigenetic marks in brain circuitry controlling the HPA or autonomic nervous system (ANS) circuitry?

In the context of human epigenetic studies, histological differences in DNA methylation pose a difficulty in two ways. First, since in many cases the tissue of interest for a particular condition may not be available (e.g. brain tissue in PTSD), post-mortem, surgical or surrogate tissues are often employed. Post-mortem tissue has been useful in some studies (e.g. McGowan, Sasaki, D'Alessio, Dymov, Labonte *et al.*, 2009), but in general, for large-scale studies, only surrogate tissue is available (Horvath, Zhang, Langfelder, Kahn, Boks *et al.*, 2012; Lowe, Gemma, Beyan, Hawa, Bazeos *et al.*, 2013). In these cases, researchers examine peripheral tissues, such as BECs or PBMCs, for associations with phenotypes manifesting in central tissues. Some have argued, for example, that the DNA methylome of BECs may be more reflective of epigenomic states in brain structures, as a consequence of their common ectodermal origin during embryogenesis (Lowe *et al.*, 2013). Since different tissues show distinctive epigenetic patterns, methylome comparisons allow an assessment of how representative central tissues may be of their peripheral counterparts. One area of particular interest is epigenetic variability, i.e. determining whether the amount of variation between individuals found in peripheral tissues matches the variation within central tissues.

Second, when comparing a specific tissue methylation profile across individuals, differences in the cellular composition of the tissue sample can substantially affect profile differences between the individuals (Jaffe & Irizarry, 2014). This can be corrected by directly measuring sample tissue composition, by using DNA methylation profiles themselves to back-predict underlying cellular composition, or by using methods that correct for composition without the actual measurements (see e.g. Houseman, Accomando, Koestler, Christensen, Marsit *et al.*, 2012; Lam *et al.*, 2012; Zou, Lippert, Heckerman, Aryee & Listgarten, 2014). Based on existing literature, it is likely that the within-individual differences in DNA methylation between tissues trumps inter-individual differences in the same tissue, rendering between-tissue differences the main drivers of DNA methylation variability (Davies, Volta, Pidsley, Lunnon, Dixit *et al.*, 2012; Lam *et al.*, 2012; Ziller, Gu, Muller, Donaghey, Tsai *et al.*, 2013).

Although the interpretive challenges of epigenetic studies in peripheral tissues are formidable, recent, non-human primate research by Provençal *et al.* (Provençal, Suderman, Guillemin, Massart, Ruggiero *et al.*, 2012) compared DNA methylation levels and sites between cells from the prefrontal cortex (PFC) and T lymphocytes in peripheral blood and found both tissue-specific differences and other methylation patterns that co-varied with early rearing conditions (mother- versus peer-reared) across cell types. Some rearing condition effects were also found only in T cells, consistent with prior observations that early adversity has an immune component unlikely to co-occur in brain. In sum, important caveats about the limitations of peripheral epigenomic measures notwithstanding, there is likely much to be learned from the epigenetic variation observable within peripheral tissues, perhaps even among human children.

Gene–environment interplay and epigenetic processes

G–E interplay has emerged as a promising point of origin in studies of divergent developmental trajectories and the emergence of mental disorder. Such interplay is

thought to comprise at least three categories of processes: (a) gene–environment correlation (rGE), (b) G×E interaction (G×E), and (c) epigenetic, chromatin modifications. First, rGE refers to a genetic biasing of environmental exposures, in the sense that individuals can be predisposed, based on genetic background, to select, alter and/or generate specific categories of experiences – e.g. the behaviorally inhibited child's choice of less challenging or intensive social environments. Second, G×E designates genetic or environmental effects on outcomes that are conditional upon each other – e.g. the effects of genetic variants becoming apparent only in the presence of specific environmental conditions, or environmental influences being revealed only among individuals of a particular genotype. Third, as discussed above, epigenetic marks controlling the structural configuration of chromatin are the means by which environmental signals guide and adjust gene transcription to maximize adaptation, fitness and health.

The exploration of these three domains of G–E interplay has become a prolific and engaging area of biomedical and social science research. Indeed, G–E interplay research holds at least implicit promise for illuminating one of the oldest and deepest mysteries of human experience, i.e. how individual susceptibility and social conditions work together – at the behavioral, physiologic, neural, cellular and molecular levels – to initiate and sustain disorders of development, behavior and health. In animal models from fruit flies (Burns, Svetec, Rowe, Mery, Dolan *et al*., 2012) to rats (Meaney, 2001) and non-human primates (Barr, Newman, Lindell, Shannon, Champoux *et al*., 2004a), new evidence has accumulated that G–E interplay plays a role in the genesis of species-typical and deviant behavior. Though such evidence had been long anticipated, it was only a decade ago that reports began to emerge documenting G×E interactions in the longitudinal prediction of human developmental and health outcomes. The papers of Caspi, Moffitt and colleagues (Caspi, McClay, Moffitt, Mill, Martin *et al*., 2002; Caspi, Sugden, Moffitt, Taylor, Craig *et al*., 2003) from the Dunedin Multidisciplinary Health and Development Study revealed statistical interactions between early environmental conditions (e.g. child maltreatment and stressful life events) and functional gene polymorphisms (e.g. the *MAOA*, monoamine oxidase A, and *5HTT*, serotonin transporter, genes) in the prediction of antisocial behavior and depression/suicidality.

Questions were raised regarding the *5HTTLPR* × stressful events interaction in a meta-analysis of reports that followed the Caspi findings (Risch, Herrell, Lehner, Liang, Eaves *et al*., 2009), and some have more recently dismissed efforts to identify significant G×E effects within the samples of modest size, where such effects have largely been found (e.g. Duncan & Keller, 2011), attributing most G×E reports to a combination of Type I error and publication bias. Other critiques of these dissenting, meta-analytic reviews, however, have pointed to selectivity in the choice of studies, unwarranted statistical assumptions, and a failure to consider the expectation of G×E interaction on biological grounds (Rutter, Thapar & Pickles, 2009). A 2010 review of 40+ studies of G×E interactions involving the serotonin transporter polymorphism revealed not only predominantly strong (though not unmixed) confirmation of the effect, but the appearance, as well, of a large number of convergent neuroscience experiments supporting the role of the short risk allele in generating neural sensitivity to negative and stressful environments (Caspi, Hariri Holmes, Uher & Moffitt, 2010). Further, in a 2011 meta-analysis by Karg *et al*. (Karg, Burmeister, Shedden & Sen, 2011) a more inclusive strategy merging study findings at the level of significance testing, rather than raw data, allowed the consideration of 54 studies; the authors concluded that there is strong evidence for the moderation of the stress–depression association by the *5HTTLPR* polymorphism.

Despite legitimate concerns for the replicability of G×E interaction reports, some such effects have been replicated in independent samples (van Winkel, Peeters, van Winkel, Kenis, Collip *et al*., 2014), and new evidence for G×E interactions continues to accrue. Zohsel *et al*. (Zohsel, Buchmann, Blomeyer, Hohm, Schmidt *et al*., 2014) reported an interaction between the 7-repeat allele of the *DRD4*, dopamine receptor gene and maternal reports of prenatal stressors in predicting risk for diagnoses of conduct disorder or oppositional defiant disorder in early adolescence. Drury *et al*. (Drury, Theall, Smyke, Keats, Egger *et al*., 2010), from the Bucharest Early Intervention Project, identified a G×E interaction in which children who remained institutionalized and were carriers of the met allele of the *COMT*, catechol-O-methyltransferase gene had significantly higher mean levels of depressive symptoms. COMT degrades catecholamine neurotransmitters, such as dopamine and norepinephrine, and has been implicated in risk for major depression. Broekman and colleagues (Broekman, Chan, Goh, Fung, Gluckman *et al*., 2011), examining socioemotional development in children from a cohort study in Singapore, found that specific allelic variants in three genes involved in serotonergic functioning (*TPH2*, *SCL6A4*, and *HRT2A*) moderated the influence of birth weight on internalizing symptoms at 8–12 years of age. SNPs in each of the three serotonin-related genes were associated with a significant reduction in symptoms, but only among children occupying the

third quartile of birth weight (i.e. the quartile immediately above the median). Further, in another form of G×E interaction detectable in twin samples, Tucker-Drob *et al.* (Tucker-Drob, Rhemtulla Harden, Turkheimer & Fask, 2011) used data from the Early Childhood Longitudinal Study to show that genetic variation in cognitive ability depends upon reciprocal, developmentally moderated interactions between child and environment, such that by age 2 years, genetic effects on Bayley Short Form scores were larger for children being raised in higher socioeconomic status (SES) homes.

Beyond these prospective, observational studies, true experimental evidence for G×E interaction has emerged from random allocation trials with non-human primates. Suomi and colleagues, for example, have demonstrated, in an experimental model with rhesus macaques how early rearing conditions, within either mother- or peer-reared groups, interact with the *5HTTLPR*, serotonin transporter promoter polymorphism to predict ACTH expression during separation stress (Barr, Newman, Shannon, Parker, Dvoskin *et al.*, 2004b). Such interactions appear to apply even in regard to the timing of normative developmental events, as shown in another rhesus model in which social dominance status during development interacted with the *5HTTLPR* polymorphism to predict the timing of sexual maturation (Wilson & Kinkead, 2008). Specifically, subordinate female monkeys carrying at least one copy of the short promoter variant had significant delays in pubertal sexual maturation.

Thus far, such experimental G×E effects have not been extended to human samples, though there are no prohibitive reasons why causal evidence could not also be derived from human groups, using study designs with random assignment by genotype. As noted by Kraemer (Kraemer, 2012), the research design and mathematical modeling difficulties inherent in detecting and interpreting the cooperation of genes and environments create a 'perfect storm' of methodological challenges. That tempest occupies the dead-center, however, of the possibly most fertile and promising arena of contemporary biomedical research: i.e. how genes and environments work together to undermine health. The search for reliable G×E interactions may be abetted by the development of both empirical evidence that polygenic risk scores associated with developmental phenotypes can discern promising new SNP targets (Rietveld, Medland, Derringer, Yang, Esko *et al.*, 2013) and computational models suggesting that genome-wide association study (GWAS) approaches to G×E discovery may be more promising than candidate SNP by SNP searches (Marigorta & Gibson, 2014). Indeed, in fields such as psychiatric genomics, the way forward appears to lie in new knowledge of how multiple genes with additive or multiplicative effects, each with incremental influences, are assembled into functional genetic networks that interact with social environmental conditions to produce important phenotypic disorders (Gratten, Wray, Keller & Visscher, 2014).

Epigenetics and the mediation of G×E interactions

Rothman and Greenland (Rothman & Greenland, 2005) have argued that, in effect, all disorders of health and development are uniformly both genetic and environmental, in the sense that virtually all endpoints depend upon mutually interactive influences of both. Among the most intriguing recent discoveries in support of such a perspective implicate *epigenetic processes* as possible molecular mechanisms for G×E at the population level. It is known, for example, that maltreatment of children bears important but complex linkages to dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) axis (Tarullo & Gunnar, 2006), to pro-inflammatory shifts in cytokine signaling pathways (Danese, Pariante, Caspi, Taylor & Poulton, 2007), and to long-term changes in epigenetic and gene expression signatures within key, stress-responsive neural structures (McGowan *et al.*, 2009). Exploring the known interaction between childhood maltreatment and an allelic variant in the *FKBP5*, FK506 binding protein 5 gene in predicting adult PTSD, for example, the Binder laboratory found a mediating molecular event involving demethylation of a CpG site within an intron of the risk allele. *FKBP5* codes for a so-called 'chaperone protein' that alters the function of the glucocorticoid receptor (GR) and impedes the translocation of the GR-glucocorticoid complex into the cell nucleus. The demethylation event, which can occur only during an early critical period and results in a persistent activation of *FKBP5*, then primes the risk allele carrier for stress-induced over-production of the chaperone protein, suppression of GR function, HPA axis dysregulation and a consequent augmentation of risk for PTSD. Though Binder's group provides compelling laboratory evidence for a molecular, epigenetic mechanism mediating the *FKBP5* × maltreatment interaction, it would be important in future epidemiologic work to estimate the portion of variance in methylation states that is attributable to the interaction. Epigenetic marks (whether DNA methylation/demethylation or post-translational histone protein modifications) are likely only one of several molecular mechanisms controlling gene expression, but this empirically driven intersection of the fields examining genetic and epigenetic variation offers one groundbreaking account of *how* and *under what conditions* G×E interactions arise.

While Klengel *et al*. shed new light on the epigenetic mechanism of a G×E interaction, findings from the Holbrook laboratory by Teh *et al*. (Teh, Pan, Chen, Ong, Dogra *et al*., 2014) make essentially the same inference, but from the reverse direction: showing how neonatal human methylomes are affected by both individual, intrauterine exposures and fixed genetic variation, i.e. G×E interactions. Specifically, surveying the methylomes of over 200 Singaporean newborns, the investigators found over 1400 punctate genomic regions that were highly variable in methylation status across individuals – locations termed variably methylated regions (VMRs). In assessing the environmental (e.g. birthweight as a proxy for nutrition, smoking, maternal depression, and maternal body mass index), genetic and G×E origins of VMRs, G×E interactions were found to account for 75% of VMRs, through a combination of intrauterine environmental signatures on the fetal epigenome and the physicochemical effects of sequence variation on a CpG site's propensity for methylation. In fact, no VMRs were best accounted for by environmental linkages alone, independent of gene sequence variation. These findings reveal an increasingly convergent picture of how genetic variation in DNA sequences, naturally occurring (and sometimes pernicious) variation in social environmental exposures, and their molecular-level interactions operate, through chromatin modifications and other transcription regulatory processes, to produce combinatorial disturbances in developmental and health endpoints.

Lastly, Kobor and colleagues, in a series of studies examining epigenetic and allelic variation within human populations, have shown that DNA methylation profiles are highly divergent between human populations and that the sources of such divergences involve differences in allelic frequencies and complex epistatic[1] and G×E interactions (Fraser, Lam, Neumann & Kobor, 2012). Examining over 14,000 genes in 180 different cell lines from European and African samples, they found population-level differences in DNA methylation near transcription start sites in over a third of genes. Further analyses indicated that these methylation differences were mostly attributable to differences in allele frequencies. Several other groups have similarly documented such epigenetic dissimilarities between human populations (Adkins, Krushkal, Tylavsky & Thomas, 2011; Heyn, Moran, Hernando-Herraez, Sayols, Gomez *et al*., 2013; Moen, Zhang, Mu, Delaney, Wing *et al*., 2013).

[1] Interactions in which the phenotypic effect of a given gene is moderated by the effects of another gene or network of genes (i.e. G×G interactions).

Taken together, these recent observations suggest that much of developmental variation, and in particular that involving risks for or manifestations of developmental psychopathology, may be linked to interactions between polygenic and environmental differences. Further, emerging evidence implicates epigenetic processes as molecular-level mechanisms by which such interactions may occur. Thus, the *statistical* interactions identified between allelic variants and risk-engendering early social environments may be rooted in and attributable to differences in DNA methylation, post-translational histone modifications, or ncRNAs – resulting in inter-individual variation in the transcription of genes linked to pathological phenotypes or endophenotypes.

An epigenetic embedding of early deprivation, adversity and developmental risk

Adversity and epigenetic marks

Among such endophenotypes are the patterns of epigenetic modifications that attend early exposures to deprivation, maltreatment and adversity. Recent evidence, from both experimental animal models and human observational studies, reveals reliable deprivation- and stress-related differences in DNA methylation and histone modification that are developmentally timed and affect stress-responsive, neuroendocrine pathways with known linkages to developmental psychopathology (Monk, Spicer & Champagne, 2012). In a set of transformative studies, for example, Meaney and colleagues (Weaver, Diorio, Seckl, Szyf & Meaney, 2004, Meaney 2010) used naturally occurring differences in maternal licking and grooming of rat pups to demonstrate how low maternal caretaking up-regulates pups' long-term HPA reactivity through decreases in hippocampal GR (*NR3C1*) expression and serotonergic tone. Maternal licking and grooming triggers increases in serotonin (5-HT) expression in the hippocampus, and activation of the 5-HT receptor induces a transcription factor, nerve growth factor-inducible protein-A (NGFI-A). Maternal care also facilitates NGFI-A's association with the exon 1_7 GR promoter by demethylating a CpG site located in the promoter's NGFI-A binding region. *Low* licking and grooming is thus linked to *increased* DNA methylation and *decreased* histone acetylation within the *NR3C1* gene, resulting in diminished expression of GR, an up-regulation of corticotropin releasing hormone (CRH) secretion, and greater activation of the HPA axis.

In other rodent work, early infant maltreatment has been linked to an enduring, increased methylation of the *BDNF*, brain-derived neurotrophic factor gene, reducing

BDNF expression (Roth, Lubin, Funk & Sweatt, 2009). Chronic, variable stress during the first trimester of pregnancy in mice results in heightened corticosterone expression and increased depressive behavior in the offspring, linkages that are mediated by sex-specific differences in DNA methylation in the *CRH* and *NR3C1* genes (Mueller & Bale, 2008). Maternal behavior, neglect and abuse by no means affect only the epigenetic states of genes involved in HPA regulation, however, as over 900 genes are differentially expressed in the hippocampus as a result of low versus high maternal care (Weaver, Meaney & Szyf, 2006). There is also experimental evidence, summarized by Korosi *et al*. (Korosi, Naninck, Oomen, Schouten, Krugers *et al*., 2012) that the effects of early life stress on adult neurogenesis in the rat may be mediated by various components of the epigenetic molecular machinery.

Evidence for early social adversity effects on epigenetic states has now extended to non-human primates, as well. Suomi and others (Provençal *et al*., 2012; Tung, Barreiro, Johnson, Hansen, Michopoulos *et al*., 2012) have shown that social dominance rankings and rearing conditions are associated with epigenetic and gene regulatory variation in the immune system of rhesus macaques. Mother- versus peer-rearing results in differential methylation patterns in the infants' prefrontal cortical neurons and T lymphocytes. Further, such patterns in response to rearing conditions were not randomly distributed across the genome but showed a structural organization targeting specific cellular functions. Cole and colleagues (Cole, Conti, Arevalo, Ruggiero, Heckman *et al*., 2012), also working with infant rhesus macaques, found up-regulated expression of genes involved in pro-inflammatory cytokine signaling and T-cell activation among peer-reared monkeys, as well as suppressed expression of other genes with roles in antimicrobial defenses. Kinnally and colleagues (Kinnally, Feinberg, Kim, Ferguson, Leibel *et al*., 2011), in a study of bonnet macaques randomly assigned to an early, stressful variable foraging demand condition, found associations among developmental exposures to feeding adversity, behavioral stress reactivity, enhanced whole genome methylation, and epigenetic modification of the *5HTT*, serotonin transporter gene.

Human studies of early adversity and neglect show parallel evidence for effects on endophenotypic epigenetic marks, in relation to both pre- and post-natal events. Periconceptual exposures to famine and adversity during the 1944–45 Dutch Hunger Winter, for example, were associated with decreased methylation in the I*GF2*, insulin-like growth factor II gene, which has an important role in developmental biology and growth (Heijmans, Tobi, Stein, Putter, Blauw *et al*., 2008), as well as differential methylation in a variety of other developmentally and immunologically active genes (Tobi, Lumey, Talens, Kremer, Putter *et al*., 2009). Other research with institutionalized children 7–10 years of age reported whole genome hypermethylation, compared to parent-reared controls (Naumova, Lee, Koposov, Szyf, Dozier *et al*., 2012). Oberlander and colleagues (Oberlander, Weinberg, Papsdorf, Grunau, Misri *et al*., 2008) reported increased *NR3C1*, GR gene methylation among infants born to mothers with high depressive symptoms during the third trimester of pregnancy, and Radtke *et al*. (Radtke, Ruf, Gunter, Dohrmann, Schauer *et al*., 2011) derived similar findings from the leukocytes of adolescents whose mothers were exposed to intimate partner violence during pregnancy. Romens *et al*. (Romens, Svaren & Pollak, 2014), in a community sample of early adolescents, found similarly increased DNA methylation of the GR promoter among children who had been physically abused, compared to peer controls, and similar results have been reported in hippocampal cells from suicide victims with a history of child abuse (McGowan *et al*., 2009; Sasaki, de Vega & McGowan, 2013). Ouellet-Morin and colleagues (Ouellet-Morin, Wong, Danese, Pariante, Papadopoulos *et al*., 2013) found more extensive DNA methylation of the serotonin transporter (*SERT*) gene among bullied monozygotic twins than in their non-bullied co-twins, and, in a sample of healthy adults, other investigators found reports of parental loss, maltreatment, and impaired parental care to be associated with GR methylation status (Tyrka, Price Marsit, Walters & Carpenter, 2012).

Despite these multiple reports of heightened, adversity-related methylation in the human and animal GR gene promoter, findings in this regard are not uniformly positive in either animals or humans. Methylation differences are often quite modest in magnitude, and confounds, such as the proportions of individual cell types in peripheral blood, have frequently not been taken into account. Witzmann *et al*. (Witzmann, Turner, Meriaux, Meijer & Muller, 2012), for example, in an early chronic stress model in rats, confirmed some DNA demethylation in the GR promoter, but in contrast to Weaver (Weaver *et al*., 2004), was unable to detect hypomethylation in the NGFI-A recognition site of the GR 1_7 promoter. In another example, Alt *et al*. (Alt, Turner, Klok, Meijer, Lakke *et al*., 2010) found that GR promoter methylation was unchanged in the post-mortem brains of patients with major depression but no early trauma, whereas McGowan and colleagues (McGowan *et al*., 2009) had reported GR promoter demethylation in the brains of individuals with histories of child abuse. It is thus possible that GR demethylation, as one example of an adversity-related epigenetic modification, is highly specific with respect to both the character of the early

adversity encountered, as well as the exposure timing (Klengel *et al*., 2014).

Nonetheless, other epidemiologic research at a more genome-wide level by Borghol *et al*. (Borghol, Suderman, McArdle, Racine, Hallett *et al*., 2012), Essex *et al*. (Essex, Boyce, Hertzman, Lam, Armstrong *et al*., 2013) and Kobor and colleagues (Lam *et al*., 2012; Powell, Sloan, Bailey, Arevalo, Miller *et al*., 2013) detected longitudinal associations between childhood disadvantage and genome-wide promoter methylation in mid-life, between parental stress in infancy and differential methylation in adolescence, and between early socioeconomic status and inflammatory gene expression in leukocyte transcriptomes. Such findings, of broad epigenomic differences in DNA methylation status, are commensurate with the known cellular effects of stress hormones, such as glucocorticoids, which are known to moderate expression of approximately 10% of the genome (Matthews & Phillips, 2012). They are supported, as well, by reports of real-time, dynamic changes in gene methylation among individuals exposed to laboratory-based, stressful challenges (Unternaehrer, Luers, Mill, Dempster, Meyer *et al*., 2012). Taken together, the observations offer credible evidence, in both animal and human systems, for a possibly extensive interplay among genes, epigenomes, and social environments in the genesis of epigenomic endophenotypes.

Psychopathology and epigenetic marks

The endophenotypes related to early adversity are of great salience to human population health because of the abundant social environmental challenges with which millions of young children contend around the globe, including poverty, war, subordination and bullying, maltreatment, parental mental illness or addiction, family dissolution, and exposures to violence in both home and community. There is now a substantial body of evidence – and a strong, corresponding scientific consensus – that such adversity and stress in early life are also associated with disturbances of childhood mental health, more disordered developmental trajectories, poorer educational achievements, and lifelong risks of chronic disorders of health and well-being (see e.g. Shonkoff, Boyce & McEwen, 2009; Hertzman & Boyce 2010; Boyce, Sokolowski & Robinson, 2012). Further, there is related evidence that such experiences of 'toxic stress'[2] are socioeconomically partitioned, that the exposures are biologically embedded into epigenetic processes potentially affecting health risks, and that broad individual differences exist, also epigenetically mediated, in the health and developmental consequences of stress.

Stress-related psychiatric conditions, such as suicidal ideation and attempts, have been associated, for example, with genetic variation in the molecular machinery of epigenetic processes, such as polymorphisms found in the *DNMT* gene (Murphy, Mullins, Ryan, Foster, Kelly *et al*., 2013). In another study of depressed adolescents and their unaffected peers, symptoms were more common among youth who showed increased buccal epithelial cell methylation in the *5HTT*, serotonin transporter gene, along with the short-allele promoter in the same gene (Olsson, Foley Parkinson-Bates, G. Byrnes, M. McKenzie *et al*., 2010). Mehta, Binder *et al*. (Mehta, Klengel, Conneely, Smith, Altmann *et al*., 2013) found distinctive patterns of whole genome expression and methylation among PTSD patients, with and without histories of childhood abuse. PTSD patients with accompanying childhood trauma exposure had 12-fold increases in DNA methylation, relative to those without such histories. There is increasing evidence for epigenetic mechanisms involved in schizophrenia, much of it focused upon hypermethylation within a large CpG island in the promoter region of the gene encoding reelin, a glycoprotein found in GABAergic neurons (Tsankova, Renthal, Kumar & Nestler, 2007). Finally, psychoactive drugs, such as cocaine and some antipsychotic agents, have been noted to induce acetylation and other modifications of histone proteins (Tsankova *et al*., 2007). In sum, studies documenting a variety of pathophysiological changes in brain and neural circuitry – including changes in regional structure and function, differences in circuitry activation, molecular dysregulation at the synaptic cleft, and alterations in intracellular kinetics and signaling pathways – may all have in common dysregulatory changes in epigenetic processes that underlie these fundamental neurobiological features.

Individual variation in epigenetic susceptibility

At the levels of both behavior and biology, however, there are dramatic differences in the consequences of exposures to early adversity, with many children showing immediate and long-term deficits in health and development, while others thrive and survive with apparent indifference to the challenges they face (Rutter, 2012; Masten, 2014). The source of this individual variation in the consequences of toxic stress has been the focus of increasing study, since understanding such differences could explain stress-related disorders, shed light on the

[2] That is, stress involving strong, frequent and/or prolonged exposures to adversity, without adequate adult support, and sufficient to activate neurobiological stress response systems (National Scientific Council on the Developing Child, 2005/2014).

sources of personal resilience and vulnerability, explain the uneven distribution of disease within human populations, and produce insights leading to more effective intervention strategies. The 'stress diathesis' perspective on such differences in stress response held that individuals varied in the impact of adversity by virtue of either heritable or acquired vulnerabilities to stress and challenge. More recently, it has become apparent that another, possibly more prevalent form of variability in contextual effects is a *differential susceptibility to environmental influence*, in which a subset of individuals appears more sensitive or 'permeable' to the influences of both negative and positive environmental factors. In a now substantial body of literature (e.g. Boyce, Chesney, Alkon-Leonard, Tschann, Adams *et al*., 1995; Belsky, 2005; Boyce & Ellis, 2005; Ellis, Boyce, Belsky, Bakermans-Kranenburg & van Ijzendoorn, 2011), differentially susceptible children (sometimes referred to as 'orchid children', in contrast to their more resilient counterparts, 'dandelion children') show either the most maladaptive or most positive outcomes, depending upon the character of their social environments.

A variety of studies have identified genetic polymorphisms that appear to function as sources of differential susceptibility. Bush *et al*. (Bush, Guendelman, Adler & Boyce, 2014, submitted), for example, have recently shown that the *BDNF* Val66Met polymorphism confers an increased neuroendocrine sensitivity to socioeconomic context, with Met-carriers having the highest and lowest cortisol expression levels, depending upon SES. Consistent with the differential susceptibility hypothesis, Belsky and Beaver (Belsky & Beaver, 2011) found that an index of risk alleles in five candidate 'plasticity' genes (*DAT1*, *DRD2*, *DRD4*, *5HTT*, and *MAOA*) moderated the link between parenting quality and male adolescent self-regulation. In a sample of several hundred African American young adults, Simons *et al*. (Simons, Lei, Beach, Brody, Philibert *et al*., 2011) found longitudinal evidence for the differential susceptibility of aggressive behavior to environmental adversity among individuals with the combination of the S-allele of the *5HTT*, serotonin transporter gene and the L-allele of the *DRD4*, dopamine receptor gene. Similarly, Babineau *et al*. (Babineau, Gordon Green, Jolicoeur-Martineau, Minde, Sassi *et al*., 2014) demonstrated differential susceptibility among children with the S- and L_G alleles of the *5HTT* gene (the latter polymorphism a variant of the L allele with a functional effect on mRNA expression similar to that of the S-allele): children with the 'risk' allele having greater behavioral and cognitive dysregulation when exposed to prenatal maternal depression, but greater regulatory capacities in the absence of such prenatal exposure. Bogdan and colleagues (Bogdan, Agrawal, Gaffrey, Tillman & Luby, 2014) also found a *5HTTLPR* × stressful life events interaction in predicting preschool-onset depressive symptoms among 3–5-year-old children. Again, those with the risk allele had either the most or least depressive symptoms, depending upon level of stressor exposure. Brett *et al*. (Brett, Sheridan, Jones, Esteves, Fox *et al*., 2014), examining data from the Bucharest Early Intervention Project, found that the high susceptibility allele of the *ERBB3*, neuroregulin gene predicted the largest corpus callosum volumes in children randomized to the foster care condition, but the smallest volumes among those remaining in orphanages. In a meta-analysis of how negative and positive rearing environments are linked to developmental outcomes, Bakermans-Kranenburg and van IJzendoorn (Bakermans-Kranenburg & van IJzendoorn, 2011) found that children with the less efficient, 7-repeat *DRD4*, dopamine receptor allele fared less well in negative environments than their counterparts without the genetic 'risk factor', but also gained most from positive rearing conditions. These findings and others indicate that allelic variation in DNA sequences can itself engender a heightened sensitivity to both negative and positive early social settings.

Within differential susceptibility theory, however, greater sensitivities to the character of the social world have also been hypothesized to *emerge* developmentally and responsively, via conditional adaptations to the social signals of early life (Boyce & Ellis, 2005; Ellis, Essex & Boyce, 2005). Conditional adaptations – such as the polyphenism in wing pattern and coloration found among butterflies emerging from chrysalises during differing seasonal conditions – are fitness-augmenting changes in development in response to early environmental cues (Gilbert & Epel, 2009; Ellis & Bjorklund, 2012; Nederhof & Schmidt, 2012). An important question is therefore whether epigenetic modifications, acquired as a consequence of early environmental signaling, might also be linked to differentially susceptible phenotypes. Suderman *et al*. (Suderman, McGowan, Sasaki, Huang, Hallett *et al*., 2012), for example, studied large, differentially methylated regions centered upon the *NR3C1*, GR gene in the hippocampi of both rats and humans experiencing substantially different levels or forms of early parental care. The methylation profiles of both species were extensively different in individuals receiving high- vs. low-level (rats) or abusive vs. non-abusive (humans) early parental care, with many between-species commonalities in the specific, differentially methylated sites. Beach *et al*. (Beach, Brody, Lei, Kim, Cui *et al*., 2014), in a sample of African American youth from working poor communities, found that cumulative socioeconomic adversity and the S-allele of

the *5HTT*, serotonin transporter gene interactively predicted promoter region methylation within a group of 200+ depression-related genes. Youth with the S-allele had either the highest or lowest levels of depression gene methylation, depending upon levels of exposure to poverty-related stress. Strunk and colleagues (Strunk, Jamieson & Burgner, 2013) have argued that the increased susceptibility of infants to infectious agents of disease may be due to differential methylation of immune-regulating and other developmentally salient genes. Finally, the Binder laboratory (Klengel, Mehta, Anacker, Rex-Haffner, Pruessner *et al*., 2013) has demonstrated a differential susceptibility of individuals bearing the AG/AA 'risk' allele of the *FKBP5* gene. Such individuals have either higher or lower rates of adult PTSD, conditional upon childhood exposures to sexual and/or physical abuse. Further, the molecular process by which this epidemiologically observed interaction occurs is mediated through DNA demethylation in the glucocorticoid response elements of *FKBP5*. These observations are among the first to show how chromatin modification and epigenetic marks may constitute actual molecular mechanisms for a differential susceptibility to environmental conditions.

Here, however, in the search for an epigenetic substrate of differential susceptibility, caution is once again essential. The putative 'plasticity genes' examined in Belsky and Beaver (Belsky & Beaver, 2011) are a salad of diverse genomic locations, serving diverse neurobiological functions. There are likely polygenic networks involved in the production of 'risk' for special susceptibilities to social contexts; such allelic variation is unlikely to operate in a single direction or be capable of generating a conserved and substantial subpopulation of exceptionally susceptible phenotypes; and because sensitivity may be context-specific (see e.g. Obradovic, Bush & Boyce, 2011), different groups of genes may be implicated in different categories of context sensitivity.

Critical periodicity, developmental time and the epigenome

Developmental time is not uniform in its potency and influence. Rather, the effects of experience change dynamically across the lifespan, especially in the early years, as critical and sensitive periods open and close. Critical periods are those in which the presence or absence of important experiences or exposures result in irreversible change in brain circuitry, while sensitive periods are developmental intervals in which the brain is especially responsive to such experience (Fox, Levitt & Nelson, 2010). Both involve experience-dependent plasticity during defined windows of early life (Takesian & Hensch, 2013). A classic example is the deprivation amblyopia that occurs in children lacking patterned visual stimulation, due to strabismus, cataracts or other occlusions of vision, during the early development of the brain's visual circuitry (i.e. birth to 7–8 years of age). As Kuzawa and Thayer noted (Kuzawa & Thayer, 2011), human adaptation to environmental conditions takes place at a variety of timescales, ranging from homeostatic changes that can occur over seconds or minutes to developmental plasticity present over months or years, to conserved genetic changes that operate on a timescale of millennia. Wright and Christiani (Wright & Christiani, 2010) point out that the critical periodicity of growth and development occurs as a consequence of the timing and sequencing of important neurodevelopmental processes, such as cell migration, synaptic proliferation and pruning, changes in receptor density and axonal myelination. There is evidence, for example, that the developing brain is especially vulnerable to the deleterious effects of chemical exposures during early developmental periods, rendering children more liable than adults to toxic injury during critical periods of neurodevelopment. Such liability also includes unique, early susceptibility to social environmental exposures, and Nelson *et al*. (Nelson, 2014) have shown, in a random-assignment trial of foster care placements for children in Romanian orphanages, how neurobiological and developmental outcomes are dramatically improved when placements occur prior to 2 years of age (Zeanah, Gunnar, McCall, Kreppner & Fox, 2011).

The molecular substrates for the occurrence of such critical periods – their openings and closings across developmental time – are being elucidated within animal models involving experimental manipulations at neuronal and molecular levels. Takesian and Hensch (Takesian & Hensch, 2013), for example, have shown how molecular 'triggers' and 'brakes' initiate and constrain plasticity in the brain over time. The persistent loss of visual acuity in amblyopia fails to occur, for example, when cortical inhibitory circuitry, formed by GABAergic interneurons (which use the inhibitory neurotransmitter gamma-amino butyric acid), is compromised. Critical period onset appears guided and timed by the maturation of excitatory–inhibitory (E–I) circuit balance. The closure of such plasticity is regulated by structural and functional molecular brakes, such as perineuronal nets and the expression of proteins such as *Lynx1*, which dampen plasticity by binding to and reducing the function of nicotinic acetylcholine receptors on specific inhibitory interneurons. Perineuronal net degradation or genetic deletion of *Lynx1* reopens a period of plasticity to restore visual acuity to adult amyblyopic animals

(Pizzorusso, Medini, Landi, Baldini, Berardi *et al*., 2006; Morishita, Miwa, Heintz & Hensch, 2010). Such findings have led to a fundamental shift in thinking about brain plasticity, from an assumption that plasticity arises during sharply defined critical periods, like square waves in the course of development, to a new understanding that the brain is intrinsically, obligatorily plastic and that normal development requires a timed, molecular suppression of that plasticity.

Much of the molecular machinery underlying critical period onset and offset is epigenetic in origin (Fagiolini, Jensen & Champagne, 2009). Epigenetic control of gene expression guides, for example, the differentiation of neurons into unique neuronal subsets, the guidance of axon growth, and the radial organization of brain development (Fox *et al*., 2010). Brain circuitry responds to environmental events by way of DNA methylation and post-transcriptional histone modifications (Takesian & Hensch, 2013). The closure of the critical period for ocular dominance acquisition, for example, involves the down-regulation of vision-dependent histone acetylation and phosphorylation. Epigenetic factors also regulate the expression of *GAD67*, the gene coding for GABA inhibitory neurotransmitter, and various pharmacological agents targeting epigenetic processes – drugs such as valproate, an HDAC inhibitor – can shift the timing of critical periods. Valproate has been shown, for example, to reopen the critical window for the acquisition of absolute pitch (Gervain, Vines, Chen, Seo, Hensch *et al*., 2013), and E–I circuitry imbalance and critical period timing errors have been recognized within mouse models of autism spectrum disorder (Gogolla, Leblanc, Quast, Sudhof, Fagiolini *et al*., 2009). These discoveries together reveal a complex critical periodicity within development – both adaptive and maladaptive – that is likely initiated, guided and curtailed by epigenetic, molecular events affecting the neuroregulatory genes that govern brain development.

Transgenerational inheritance of epigenetic marks

There is now substantial evidence in both humans and animals that adverse physical and psychological exposures in one generation can be replicated among or transmitted into subsequent generations and that such transgenerational diffusion can alter risks for psychopathology and maladaptive behavior in offspring (Franklin, Russig, Weiss, Graff, Linder *et al*., 2010). Epidemiologic studies in human populations sustaining historic generational adversities or existential threats, for example, have documented the elevated rates of psychiatric disorders in the generation that follows, despite an absence of actual exposure (Yehuda, Halligan & Bierer, 2001). At the level of stress physiology, prenatal exposures to stressors in both animal and human mothers have been associated with differences in autonomic and adrenocortical responses to challenge in offspring, though such effects substantially vary with exposure timing, offspring sex and other factors (Matthews & Phillips, 2012). These recapitulations of disorders or risk factors within new generations have been presumed in humans and demonstrated in mice to be mediated by epigenetic marks transmitted to offspring (e.g. Saavedra-Rodriguez & Feig, 2013).

Such cross-generational propagation of epigenetic marks is believed to occur via one of two possible mechanisms: either through behavioral and social transfer of such marks to the subsequent generation or through germline transmission. Though the latter is far more difficult to prove, there is evidence for both, at least in animals. The rat experiments of Meaney and colleagues is the now classical example of behavioral/social transmission of marks. In that model, abundant maternal care, indexed by high levels of maternal licking and grooming of pups, changes DNA methylation throughout the genome (McGowan, Suderman, Sasaki, Huang, Hallett *et al*., 2010) and at a transcription factor binding site in the hippocampal GR promoter (Weaver, La Plante, Weaver, Parent, Sharma *et al*., 2001). The maternal care-associated hypomethylation of the GR promoter increases expression of the receptor protein, thereby down-regulating HPA reactivity and producing stress resilience in offspring. These effects and the altered methylation status are passed on to subsequent generations through the mechanism of maternal behavior, the down-regulated reactivity predisposing the new generation of mothers to their own high levels of pup licking and grooming. In presumed, human analogs of the same behavioral/social transmission of acquired epigenetic marks, studies have documented differences in stress reactivity among the offspring of mothers affected by the Dutch Hunger Winter and alterations in cortisol expression in children who were *in utero* during the 2001 World Trade Center collapse (Matthews & Phillips, 2012).

There is also emerging evidence, however, that some alleles in germ cells can show meiotic heritability, allowing epigenetic marks to be directly propagated through gametes and challenging the doctrine that DNA sequences are the exclusive, heritable arbiter of phenotype (Whitelaw & Whitelaw, 2006). Most DNA methylation, as noted above, is erased during early embryogenesis, ensuring the pluripotency of the embryo, but recent exceptions have been reported that allow the intergenerational transmission of methylated DNA,

post-translational histone modifications, and small, non-coding RNAs (Bohacek *et al*., 2013). Reprogramming in the germline related to environmental exposures has now been demonstrated in plants, fruit flies and mammals (Franklin *et al*., 2010). These findings indicate clear, but still developing evidence for both forms of transgenerational inheritance of epigenetic risk, with the strongest findings in animal models.

Other possible epigenetic mechanisms for the conveyance of parental adversity from one generation to the next (in human and animal models) are the dysregulation of stress response pathways by the developing placenta (Bale, Baram, Brown, Goldstein, Insel *et al*., 2010; Bale, 2011), the transmission of small ncRNAs in the sperm of a traumatized father (Gapp, Jawaid, Sarkies, Bohacek, Pelczar *et al*., 2014), and an epigenetically mediated transfer of fear-conditioning by an olfactory signal (Dias & Ressler, 2014). Recent work by Yehuda and colleagues (Yehuda, Daskalakis, Lehrner, Desarnaud, Bader *et al*., 2014), moreover, has demonstrated, in the adult offspring of Holocaust survivors, differential methylation of the exon 1_F promoter of the GR gene (the human homologue of exon 1_7 in the rat) according to the PTSD status of the mother and father. Some or all of these epigenetic mechanisms may be eventually implicated in human transgenerational inheritance as the science of such transmission grows and matures.

Conclusions

The research summarized here arguably presages a period of remarkable progress in understanding the convergence of genetic and environmental variation in the genesis of typical and adversity-related, atypical development. The epigenetic processes that appear to mediate such convergence comprise a broad, complex set of molecular points of connection between the experiences of early life and the proclivities, capacities and risks encoded within the individual human genome. The adversities inherent within environments of poverty, neglect and trauma are transduced into molecular events controlling the expression of neuroregulatory genes, which in turn guide brain development, calibrate stress reactivity, and influence lifelong risks of psychopathology and other morbidities. Similarly, positive early environments of nurturance, care and stability provide an anticipatory programming of many of the same genes, thereby diminishing mental health risks, optimizing neurodevelopmental preparation for learning, and ensuring normative socioemotional development. Increasingly, the epigenetic marks and modifications controlling gene expression are being recognized as molecular mechanisms that may underlie G×E interaction effects on developmental and mental health outcomes. Although such findings are clearly provisional at present, epigenetic mechanisms constitute a genuine, physical nexus between environments and genes, between nurture and nature, between the exterior and interior determinants of human development and psychological well being.

Beyond this principal conclusion, derived from a rapidly emerging field on the frontier of human biology, this paper has highlighted several specific observations that together trace a growing, if yet hazy, perimeter of developmental epigenetics. Among these are the following:

- The epigenome is a structural overlay of genomic, chemical 'markings', which target DNA and histone proteins, alter the physical packaging of chromatin, and regulate the expression of genes, without altering the nucleotide sequence itself; epigenetic processes, in embryologic development, are the mechanisms for an enduring differentiation of cells into histologically distinctive cell lines.
- Experiences and environmental exposures, especially those in early life, can also result in the placement or removal of epigenetic marks, thereby regulating the neurodevelopment that underlies learning, behavior and risks for compromised mental health.
- These contrasting ontogenetic roles – cell line differentiation and a 'recording' of contextual experience – thus result in an 'epigenetic paradox', in which the same epigenome becomes the origin of both the longitudinal *stability* of the body's cellular structure and its moment-to-moment *plasticity* in response to environmental events.
- The G×E interactions increasingly documented within the developmental and mental health literature are likely mediated, in part, through epigenetic events that allow gene effects to be contingent upon experience and experiential influences to be conditional upon allelic variation; the epigenome thus serves as a buffer to the extremes of both genetic and environmental variation.
- Within virtually every contemporary society, the developmental and health effects of early exposures to adversity and stress are socioeconomically partitioned, with children from the lower ranks of social class sustaining greater and more severe threats to normative development; many of these pervasive SES influences on adversity-related, maladaptive outcomes are almost certainly epigenetically mediated.
- In addition to the well-documented main effects of childhood stress on health and development, there are readily observable individual differences in the

consequences of such exposures. A relatively small subset of children appear differentially susceptible to the character of their rearing environments, sustaining exceptionally poor outcomes in contexts of adversity and threat, but unusually positive outcomes in nurturant, supportive settings; there is evidence that this *differential susceptibility to social environmental influence* is also epigenetically mediated.
- Environmental influences are modulated by critical periods in development, when neurobiological circuitry is especially responsive to experience and plasticity is most accessible; the opening and closing of critical and sensitive periods are regulated by epigenetic events that guide the maturation of excitatory and inhibitory neural circuitry and the expression of molecular 'brakes' that reverse the brain's inherent plasticity.
- Epigenetic processes are also the candidate mechanisms for the transmission of risk and disorder from one generation to the next; such transmission appears to occur either through transgenerational replications of behavioral risk and protective factors or through germ line transfers of epigenetic marks.

The implications of these emerging findings are legion within the domains of developmental science and medicine. First, the advent of pharmacologic agents capable of altering basic epigenetic processes holds the promise of entirely new classes of medication for psychiatric disorders (Menke, Klengel & Binder, 2012) and developmental disabilities (Miyake, Hirasawa, Koide & Kubota, 2012). HDAC inhibitors, for example, have been shown to mimic the effects of antidepressants in socially stressed mice (Covington, Maze, LaPlant, Vialou, Ohnishi *et al*., 2009), and epigenetically active agents have begun to be used in the treatment of human mental disorders and other complex diseases (Ptak & Petronis, 2008). Epigenetic processes are accessible, as well, by way of dietary interventions that can provide or alter bioactive components of food or the microbiota (Shenderov & Midtvedt, 2014). Second, measured epigenetic variation might usefully serve as a proxy for unmeasured environmental influences, as in the report by van IJzendoorn and colleagues (van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach & Philibert, 2010), where differential methylation, a stand-in for environmental adversity, interacted with *5HTTLPR* allelic variation in the prediction of unresolved loss or trauma. Third, epigenetic biomarkers might also usefully serve as closely proximal indicators of success or failure within new therapeutic or intervention strategies, as well as signals of the causal pathways by which such successes are achieved. Schneider and Prvulovic (Schneider & Prvulovic, 2013) have suggested, for example, that DNA methylation profiles among genes with known linkages to psychopathology could be useful as epigenetic biomarkers of Major Depressive Disorder. Finally, the inclusion of epigenetic measures in studies of how genes and environments work together to affect developmental outcomes may also provide insights into why either or both such etiologic factors may appear to have effect sizes that are modest in magnitude (Kraemer, 2012). In the setting of a true, cross-over, G×E interaction, for example, the association of both genetic and environmental measures may appear vanishingly small, while the effect of the interaction term is substantial and unattributable to chance. In such a case, measurement of chromatin modifications within the subsets of participants defined by interaction categories may substantiate an inference that certain combinations of genes and environments are linkable to differences in gene expression and thus phenotype. Epigenetic differences might be legitimately viewed, in such a setting, as an endophenotypic precursor of symptoms or disorder (Flint & Munafo, 2007).

The reflexive exhilaration that attends the opening of whole new arenas of scientific investigation has been an especially visible (and contagious) aspect of the epigenetic 'bubble' currently driving the bioscientific economy. The epidemic proliferation of epigenetic publications and reports, with which this review began, is testimony enough to the excitement with which the industry of science has greeted this field. The euphoria should be tempered, however, by the limiting realities that also characterize the rapid appearance of new fields and new technologies. As recently summarized by Mill and Heijmans ((Mill & Heijmans, 2013), the emerging field of epigenetic epidemiology is a veritable *minefield* of opportunities for statistical error, misinterpretation of findings, and fallacious inference. Among the pitfalls that characterize this nascent research territory are these:
- mistaking the substantial, random variation in epigenetic marks for systematic differences attributable to environmental exposures;
- conflating peripheral and central tissue methylomes, by assuming that chromatin marks found in peripheral tissues will also be present in central tissues, such as brain;
- failing to recognize and adjust for variables, such as age, development and gender, that may confound associations between exposures and epigenetic marks;
- employing admittedly practical and useful tools, such as the Illumina 450K Human Methylation array, which targets less than 2% of the CpG sites in the

human genome and may over-focus on promoter regions and CpG islands;
- using either too inclusive or too conservative data analytic strategies, with their respective effects on estimates of Type I error;
- focusing on DNA methylation because of its accessibility and ease of measurement, to the exclusion of either other chromatin marks with more reliable linkages to gene expression or novel marks with as yet unknown functional roles;
- ignoring DNA sequence polymorphisms that can alter the likelihood or effects of chromatin modifications; and
- inadequately attending to the biological consequences of marks and their possible clustering within functional groups identifiable through genome annotations (see e.g. Bock, 2012).

Further, not all differential gene expression is mediated by epigenetic modifications, as shown by Alt *et al.* (Alt *et al*., 2010), where the expression of GR in different areas of post-mortem human brain was attributable to differential expression of the NGFI-A transcription factor, rather than DNA methylation. Not only do epigenetic processes not explain all differential gene expression, but even for those questions where it should provide tractable answers, it has sometimes failed. For example, although a variety of studies have promisingly documented a longitudinal divergence in epigenetic marks among monozygotic twins, suggesting a possible epigenetic substrate for twin discordance (see e.g. Fraga, Ballestar, Paz, Ropero, Setien *et al*., 2005), a recent paper by Baranzini and colleagues (Baranzini, Mudge, van Velkinburgh, Khankhanian, Khrebtukova *et al*., 2010) reported no epigenetic or transcriptome differences in a small sample of monozygotic twins discordant for multiple sclerosis. Such findings, even on a small scale, urge caution and restraint in the interpretation (and celebration) of the epigenetic juggernaut. There remains much unknown about how genes and environments converge in effects, how chromatin modification is linked to gene expression, and how the developmental transcriptome determines phenotypic ends. Epigenetic biology is thus a field filled with an early harvest of appealing but preliminary findings – a field that should be regarded, at present, as both 'fertile' and 'fetal'.

Such scientific realities notwithstanding, there is much to admire in the heuristic productivity of early epigenetic research in human populations. In arguably no more than a decade, the field has progressed from a tentative documentation of G×E interactions, to theoretical expositions on how such interactions might occur, to real evidence that molecular mechanisms, involving verifiable chromatin modifications, actually explain epidemiologically observed transactions between genes and environments. Developmental science is thus poised on the cusp of a truly new molecular account – at the very 'synapse' between genes and contexts – of the enormous and consequential human differences in development and mental health. There is no shortage of epigenetic *terra incognita* yet to explore, and though each generation of biologists may be predisposed – by its own genes and contexts – to the same lofty and hopeful claims for its science, it is a brilliant and formidable time, even now, to be exploring.

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