

NEUROSCIENCE FOREFRONT REVIEW

NEUROEPIGENETICS OF STRESS

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Abstract—Stress, a common if unpredictable life event, can have pronounced effects on physiology and behavior. Individuals show wide variation in stress susceptibility and resilience, which are only partially explained by variations in coding genes. Developmental programming of the hypothalamic–pituitary–adrenal stress axis provides part of the explanation for this variance. Epigenetic approaches have successfully helped fill the explanatory gaps between the influences of gene and environment on stress responsiveness, and differences in the sequelae of stress across individuals and generations. Stress and the stress axis interacts bi-directionally with epigenetic marks within the brain. It is now clear that exposure to stress, particularly in early life, has both acute and lasting effects on these marks. They in turn influence cognitive function and behavior, as well as the risk for suicide and psychiatric disorders across the lifespan and, in some cases, unto future generations. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: histone modifications, DNA methylation, HPA axis, corticosteroids, non-coding RNA, developmental programming.

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Abbreviations: 5-HT, Serotonin; ACTH, adrenocorticotrophic hormone; AVP, arginine-vasopressin; BDNF, brain-derived neurotrophic factor; BLA, basolateral amygdala; COMT, catechol-O-methyltransferase; CpG, cytosine-guanine dinucleotides; CRH, corticotropin-releasing hormone; CSD, chronic social defeat; DCS, D-cycloserine; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GC, glucocorticoid; GR, glucocorticoid receptor; H3K4, H3 lysine 4; H3K9, H3 lysine 9; HATs, histone acetyltransferases; HDACs, histone deacetylases; HDMs, histone demethylases; HMTs, histone methyltransferases; HPA, hypothalamic–pituitary–adrenal (HPA); HT, hypertonic saline solution; lncRNA, long non-coding RNA; LTM, long-term memory; Met, methionine; miRNAs, microRNAs; MR, mineralocorticoid receptor; MS, maternal separation; NaBu, sodium butyrate; NMDAR, N-methyl-D-aspartate receptor; OXTR, oxytocin receptor; piRNA, piwi-interacting RNA; POMC, proopiomelanocortin; PTSD, post-traumatic stress disorder; PVN, paraventricular nucleus; SC, skin conductance; snoRNA, small nucleolar RNA; SNP, single nucleotide polymorphism; TEs, transposable elements; Val, valine; VPA, valproic acid.

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INTRODUCTION

Stress is a common, if unpredictable, life event, and can have both adaptive and maladaptive consequences for an organism. In the natural environment, many stressors can have profoundly negative, even lethal, consequences and for this reason organisms require the capacity to rapidly and effectively adapt to stressful circumstances. They also need to keep account of lessons learned with regard to stress in terms of memories as well as behavioral and physiologic adaptations. This requirement for rapid, yet persistent, change is a challenge both to the largely fixed genome and to a brain primarily comprised of terminally differentiated neurons. Mammalian brains meet this challenge in a variety of interrelated ways, from structural and functional plasticity to epigenetic reprogramming of neural genomes.

Epigenetics is most broadly defined as transmission of information above the level of DNA sequence. The term ‘epigenetic’ has evolved substantially since its coinage decades ago and it now encompasses a range of effects from behavioral or even cultural transmission of information across and within generations down to the molecular modifications of nucleic acids and their

packaging proteins. While one might defend making a hard distinction for molecular epigenetics for the latter set of phenomena, it is not clear where the line between the molecular and what it is tempting to call behavioral epigenetics, properly lies. This is clear even in what is perhaps the most famous case of epigenetic effects in the nervous system, maternal transmission of stress responsiveness described by Meaney and collaborators (see below) where maternal behavior and molecular mechanisms are both demonstrably involved. A more useful distinction might be between the acute changes in epigenetic marks, which contribute to short-term defense of homeostasis and longer term responses to environmental change that might be regarded as allostatic, to borrow a term from the stress literature (McEwen and Wingfield, 2010). Allostasis, is defined in contrast to homeostasis as “maintaining stability through change” (McEwen and Wingfield, 2003). Homeostatic epigenetic alterations would return to a baseline level over a fairly brief period of time (say hours to days), while allostatic changes might persist through the lifespan and into future generations in the absence of a countermending perturbation. It suffices to say that this is not a definitive distinction, but it is clear the field is presently large enough that some more precise terminology is wanting.

The study of neuroepigenetics in general and the neuroepigenetics of stress has undergone substantial growth over the last decade (Hunter et al., 2013; Sweatt, 2013; Reul, 2014). This is due to both the adoption of novel technical approaches such as next generation sequencing, and to the clear need to explain persistent, environmentally sensitive behavioral variations that were not due simply to genetic polymorphisms (e.g. the “Missing Heredity” problem in psychiatric genetics (Crow, 2011; Danchin et al., 2011)). Stress has long been known to play a role in brain plasticity (Hunter et al., 2013; Hunter and McEwen, 2013). As stress is one of stronger environmental influences on human and animal behavior, it is a logical means to examine genome–environment interactions with an epigenetic lens (with the caveat that the distinction between environmental, genomic and other stochastic factors is, if anything, less clear in the present age than previously).

STRESS AND THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

Stress can be conceived of as any threat to bodily homeostasis—real or imagined—that urges the organism to act in defense of that homeostasis. These actions often require behavioral or physiologic changes on the part of the organism and are therefore referred to as allostatic (McEwen and Wingfield, 2003), while the stressor itself represents an allostatic load. Stressors are defined both by their duration as well as by the capacity of the organism to respond to them. Controllable stressors, such as voluntary exercise, can be both physically and psychologically beneficial (Adlard and Cotman, 2004; Aschbacher et al., 2013). Opinion varies on whether such events are properly called ‘stress’, though they do actuate many of the same physiologic

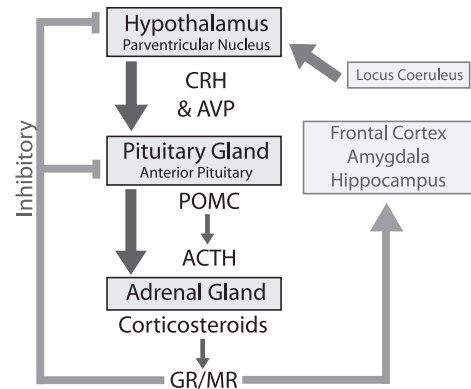


Fig. 1. The HPA axis. Stress triggers a cascade of signaling in the hypothalamic–pituitary–adrenal (HPA) axis. The hypothalamus releases corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) into the pituitary portal. The pituitary converts proopiomelanocortin (POMC) to adrenocorticotrophic hormone (ACTH) and releases it into the bloodstream, where it binds to the adrenal gland. Corticosteroids released by the adrenal gland bind to glucocorticoid (GR) and mineralocorticoid (MR) receptors. These receptors inhibit the HPA axis influencing other brain regions and serotonin receptors (5-HTR).

mechanisms. In contrast, in situations where the organism does not have meaningful control of the outcome of the stressor, the effects can be negative (Maier and Watkins, 2010). This is true of both severe acute traumas, which can induce Post-Traumatic Stress Disorder (PTSD), and of more chronically aversive situations such as social subordination or chronic unpredictable stress, which can contribute to depressive behavior. In a successful stress response, once the individual has escaped the situation, the body will return to a pre-stress state. In these instances, stress is a positive response that keeps the individual alive and well, and can even increase resilience to future stressors. Both negative and positive stress adaptations cause the nervous system to undergo epigenetic changes that influence its future responses.

The mammalian stress response is orchestrated, in part, by the activity of the hypothalamic–pituitary–adrenal (HPA) axis (Fig. 1). Perturbation of glucocorticoid (GC) feedback in the HPA axis is one of the best-established biomarkers for a number of complex diseases, including depression and PTSD. The hypothalamus is the first structure in this common pathway. Activation of the parvocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus induces three distinct responses. Activation of the pathway begins with the release of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) into the pituitary portal. This triggers the next step in the HPA cascade. CRH and AVP release into the pituitary portal increases the production of proopiomelanocortin (POMC) in the anterior pituitary. POMC is converted to adrenocorticotrophic hormone (ACTH), which is then released into general circulation in the blood (Aguilera, 2012).

The adrenal gland produces corticosteroids (predominantly cortisol in humans, corticosterone in rodents) in the presence of ACTH. Corticosteroids bind to mineralocorticoid (MR) and glucocorticoid (GR)

receptors. In the PVN and pituitary GRs have an inhibitory effect, and this negative feedback closes the loop and represents the main mechanism of restoring the HPA axis to baseline levels after a stress response. MR and GR in other brain regions, such as the hippocampus, amygdala and prefrontal cortex, also contribute to the modulation of the HPA axis, spatial cognition and anxiety behavior (Rozeboom et al., 2007; Harris et al., 2013). Other portions of the nervous system also mediate responses to stress, in particular, the locus coeruleus has noradrenergic projections directly to the PVN (Sawchenko and Swanson, 1981). Further, the autonomic nervous system also plays a significant role, releasing catecholamines into the blood and tuning down housekeeping activities like digestion, while increasing physiologic responses favorable to fighting or fleeing.

The developmental programming of the HPA axis in response to stress has been documented for decades (Liu et al., 1997), although understanding the mechanisms for these stable behavioral modifications remains incomplete.

EPIGENETIC MECHANISMS

The term “epigenetic” was initially coined by Waddington to describe how organisms were able to selectively modulate a fixed genome to develop a variety of tissues and cell fates (Waddington, 1942). The meaning of the term has broadened substantially since the 40’s to encompass non-genetic transgenerational inheritance, as well as a number of specific molecular mechanisms that influence the transcriptional phenotype at a level above that of the DNA sequence. Epigenetic information can be passed on to subsequent generations through fetal programming, behavioral intervention or germline transmission (Bohacek and Mansuy, 2013). Stress influences each of these types of inheritance, and epigenetic mechanisms can be used to explain the known developmental and transgenerational programming of the HPA axis.

Cells have several methods of regulating the expression of genes in response to stress without actually changing the code of DNA itself. These include both the epigenetic mechanisms we outline below

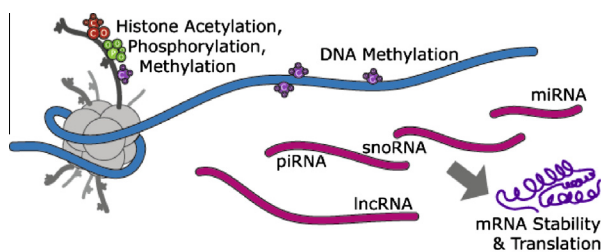


Fig. 2. Epigenetic marks. Epigenetic modifications include covalent modifications of histone proteins including acetylation, methylation, and phosphorylation; methylation of DNA, as well as a suite of mechanisms such as higher order chromatin structure and non-coding RNA (e.g. microRNA (miRNA), piwi-interacting RNA (piRNA), small nucleolar RNA (snoRNA) and long non-coding RNA (lncRNA)), which can affect the translation and stability of messenger RNA (mRNA).

(Fig. 2) and the classical transcription factors. At the molecular level, the line between epigenetic mechanism and the transcriptional machinery is not easily established, as the two processes are intimately intertwined, both structurally and functionally. Most of following mechanisms adjust the likelihood of a gene being exposed to transcriptional proteins, but there are several post-translational mechanisms as well (see ‘Non-coding RNA’).

Gene expression and epigenetic marks are cell and tissue specific, and changes observed in one area of the brain may not be the same in another brain structure (Davies et al., 2012). Likewise, epigenetic changes in the brain may not be the same as those found in blood (Axelsen et al., 2007). Because of the difficulties of examining living human brain tissue, most experiments in humans use blood to observe epigenetic differences. The correlation between changes seen in the blood, and those found in the brain is still unclear. Any test for human stress disorders will need to utilize epigenetic data from peripheral tissues to be a viable. However, because stress induces an endocrine response which reaches most tissues, it may affect the brain and peripheral tissues in a similar manner. A recent study found DNA methylation of *Fkbp5* was correlated in the dentate gyrus and blood, though the pattern was not precisely matched (Ewald et al., 2014).

DNA modification

Perhaps the most-studied form of molecular epigenetic modification is DNA methylation. Methylation occurs on the cytosine base of DNA, and most often on cytosine-guanine dinucleotides (CpG). Generally this decreases the likelihood that a gene will be transcribed, though methylation can have a more complex role in transcription (Jones, 2012). CpG repeats are not equally distributed throughout the genome, instead being located mainly within CpG islands. The presence of a CpG site in a gene represents a greater probability of that gene being methylated, and methylation can happen in the promoter, exon, or introns of genes, in that relative order of commonality (Gelfman et al., 2013).

DNA methylation in mammals is regulated by methyltransferases DNMT1/3a/3b. All three are highly expressed during postnatal development in rats, and then decline after 3 weeks, but are still expressed in mature rat neurons (Miller and Sweatt, 2007; Simmons et al., 2013). DNMT1 and DNMT3a have been shown to be active in adult mouse neurons, and loss of function impairs learning and memory (Feng et al., 2010; Maddox et al., 2014). DNMT3a is required for *de novo* methylation of non-CpG cytosine bases in maturing mouse neurons (Guo et al., 2014) and emotional regulation (LaPlant et al., 2010). Demethylation is currently less well understood, with DNA repair mechanisms, GADD45a and TET family proteins being non-exclusive suspects (Guo et al., 2011; Niehrs and Schafer, 2012; Delatte et al., 2014). As yet the former two mechanisms have yet to be explored in the context of stress neuroepigenetics, while some suggestive work has been done with the TET proteins. Knockdown of TET1 results in increased

methylation of brain-derived neurotrophic factor (BDNF) in the mouse brain (Turner et al., 2010). BDNF is required for normal brain development and plasticity, and its dysregulation has been noted in several disorders (Castren and Rantamaki, 2010). There is also evidence that GRs can influence DNA methylation through their binding to GC response elements (Thomassin et al., 2001). Hydroxymethylation is a recent addition to the list of known epigenetic mechanisms, and although its exact role is not quite clear, early evidence suggests it plays an important role in brain development, and is part of the TET demethylation pathway (Kriaucionis and Heintz, 2009; Wu and Zhang, 2011; Wang et al., 2012).

Histone modifications

Histones are the main protein constituents of the nucleosome, the fundamental packing unit of chromatin. Nucleosomes are an octamer assembled from four core histone proteins: H2A, H2B, H3, and H4. Densely packed and highly methylated chromatin forms transcriptionally silent heterochromatin, while euchromatin is less tightly packed and has higher levels of histone acetylation. Euchromatin is the site of most active gene transcription. The histone subunits each have an unstructured N-terminal tail, which may be modified to influence other proteins' ability to bind and transcribe DNA. The number of possible modifications to histones is quite large, and beyond the scope of this paper (Allis et al., 2007; Tan et al., 2011), as most neuroepigenetic research focuses on three main marks: methylation, acetylation, phosphorylation (Jiang et al., 2008; Hunter, 2012). Therefore we will focus on these marks.

Methylation of histone tails occurs on lysine bases, which may be mono-, di-, or tri-methylated, or arginine bases, which may be mono- or di-methylated (Kouzarides, 2007). Histone methylation is considered a relatively stable and specific mark, and may either increase or decrease gene expression, depending on the specific histone subunit, base residue, and methylation valence. Histone methyltransferases and demethylases (HMTs and HDMs) show a relatively high degree of specificity with regard to histone subunit and amino acid residue (Shi et al., 2004). For example, H3 lysine 4 (H3K4) methylation is transcriptionally active (Strahl et al., 1999), whereas gene silencing occurs from the di- or tri-methylation of the H3 lysine 9 (H3K9; Lachner et al., 2001) or H3K27 (Cao et al., 2002). The histone demethylase LSD1 acts on H3K4 and is important for neural differentiation (Sun et al., 2010) and present in adult rat brains (Zhang et al., 2010). Histone methylation can vary widely in response to stress, with several histone subunits becoming more methylated, and others less methylated (e.g. Hunter et al., 2009).

Histone acetylation generally increases gene expression by increasing access to genes (Tsankova et al., 2004). Histone acetyltransferases (HATs) increase histone acetylation, and histone deacetylases (HDACs) decrease acetylation of histones and other proteins (Yang and Seto, 2007). It naturally follows that HAT inhibitors decrease histone acetylation and HDAC inhibitors increase histone acetylation, and both are drugs of

interest for treating stress disorders in different brain structures. HDAC inhibitors can also contribute to the demethylation of DNA (Cervoni and Szyf, 2001). Unlike the specificity of histone methylation, HATs and HDACs have a broad influence on the entire genome. However, acetylation control can be linked to other histone marks. Stress-induced H3K14 acetylation and H3S10 phosphorylation appear to involve actions of GR on the extracellular signal-regulated protein kinase-mitogen activated protein kinase (ERK-MAPK) signaling pathway, and this pathway in turn activates the nuclear kinases MSK1 and Elk-1 leading to histone phosphor-acetylation and altered gene expression in the rat dentate (Gutierrez-Mecinas et al., 2011).

Phosphorylation is a common method of regulating the activity of proteins in the cell, and can be used to modify histones as well. Most of what is known about histone phosphorylation relates to the cell cycle (Nowak and Corces, 2004), but it has been shown that histones are differentially phosphorylated in response to stress (Trollope et al., 2012; Rotllant et al., 2013). Neural activity can result in phosphorylation of histones in postsynaptic neurons (Crosio et al., 2003), and forming memories during stressful events is dependent on histone phosphorylation (Reul and Chandramohan, 2007). An important role of histone phosphorylation may be the effect of cross-talk with other histone modifications (Ruthenburg et al., 2007; Banerjee and Chakravarti, 2011). Much more work is needed to determine the role histone phosphorylation plays in stress and stress disorders.

Non-coding RNA

Classically, RNA has been thought of as the product of a transcribed gene that is eventually translated into a functional protein. However, there are many RNAs that do not encode gene products, but still play a regulatory role in gene expression. MicroRNAs (miRNAs), perhaps the best known, are present at all stages of neural development, and contribute to mature neuronal function (Sun et al., 2013). Their expression can be controlled by behavior, and has been implicated in many mental disorders (Eacker et al., 2013). Other non-coding RNAs known to play a role in behavior include piwi-interacting (piRNA; Landry et al., 2013), long non-coding (lncRNA; Fenoglio et al., 2013), and brain-specific small nucleolar RNA (snoRNA; Cavaille et al., 2000). Non-coding RNA represents a potential wealth of mechanisms for stress disorders, and has only just begun to be studied.

Other mechanisms and potential influences on neuroepigenetic marks

Several additional mechanisms, which may either be regarded as potentially epigenetic or which interact with epigenetic mechanisms, may play a role in the brain's response to stress, but need further elucidation. Prions have been implicated in the persistence of memory (Kandel et al., 2013), and synapse stability (Blaze and Roth, 2013; Heinrich and Lindquist, 2011). Chromatin loops are supranucleosomal organizational structures

which can have an effect on gene regulation (Kadauke and Blobel, 2009), and which may be associated with the pathogenesis of Rett syndrome (Horike et al., 2005). Long thought of as “junk” DNA, transposable elements (TEs) are potentially mobile genomic constituents, which have long been thought to become active in response to stressors (McClintock, 1951, 1984; Faulkner et al., 2009). Transposition appears to play a role in both nervous system development and in adult neurogenesis (Gage and Muotri, 2012). Recent work has shown that TEs are actively transcribed in brain and that their RNAs can have both gene regulatory and pathogenic effects (Reilly et al., 2013) and that they can be dynamically regulated by environmental stress via histone modification (Hunter et al., 2012), in addition to more static regulation by both DNA and histone methylation. Indeed it has been argued that at least some of these elements may provide for increased phenotypic plasticity in response to environmental insults (Hunter et al., 2013; Sharif et al., 2013). The role of nutrition and the effects the microbiome may exert on the central nervous system have seen an increase in interest with regard to their potential effects on epigenetic marks in the nervous system (Burdge and Lillycrop, 2010; Stilling et al., 2014). DNA methylation, histone modification, and non-coding RNA are the best described mechanisms of molecular epigenetic action, but the above examples show that new sorts of molecular machinery have the potential to be added to the list in the near future.

DEVELOPMENTAL STRESS

Stress during development can change how an individual will respond to stress in the future, through all periods of life. Recent years have seen the conclusive demonstration that childhood adversity has profound, life-long effects on both physical and mental health (Larkin et al., 2014). While manipulations such as maternal separation can result in a hyper-responsive HPA axis, more mild stress can actually increase resiliency and temper some of the effects of later life stress (Francis et al., 1999; Francis and Meaney 1999; Liu et al., 2000; Champagne et al., 2003). Learning to manage stress in early development through mild exposure is likely needed for learning to cope with stress as an adult (DiCorcia and Tronick, 2011).

Animal models of developmental stress

This effect is most classically associated with mother rats' pup-grooming behavior. Rat pups with mothers less receptive to nursing and less active in grooming have lower levels of GRs in the hippocampus and elevated stress responses in adulthood (Liu et al., 1997; Francis et al., 1999). The main mechanism of these epigenetic changes was the mother's behavior, which was shown to increase DNA methylation and decrease histone acetylation of the GR promoter 1–7 in the rat hippocampus, which led to decreased binding of the transcription factor, NGFI-A (Weaver et al., 2004, 2007). Switching the pups of lessattentive mothers to highlyattentive mothers produced the same phenotype as the highlyattentive

mother's biological offspring, providing strong evidence that inflated responses to stress are not genetically determined, but programmed sometime during development.

Maternal separation (MS) is a model of developmental stress in rodents in which the mother is removed from her newborn pups for several hours. Male MS mice exhibited strain-specific differences on behavioral tests and in epigenetic marks. Though both strains had hypermethylation of AVP, only C57BL/6J mice had hypomethylation of the transcription factor *Nr4a1*, and only DBA/2J mice showed hypermethylation of GR (Kember et al., 2012). Human adults who had experienced childhood trauma also had hypermethylation of the GR promoter (Tyrka et al., 2012).

MS resulted in sex-specific differences in the methylation of the CRH promoter (Chen et al., 2012). When measured at 60 days, both female and male rats showed increased ACTH and corticosterone levels in response to restraint stress. Female MS rats had higher basal levels of corticosterone. Both females and males had higher levels of CRH messenger RNA (mRNA) in the hypothalamus after 30 min of restraint. In parallel with the corticosterone findings, female MS rats had higher basal levels of CRH mRNA. These alterations were correlated with hypomethylation of the CRH promoter region in the PVN of the hypothalamus.

BDNF has been consistently associated with stress-induced neuroplasticity throughout development (Gray et al., 2013a,b). Thus, it is of interest that BDNF is subject to epigenetic regulation. For example, MS rats had lower levels of BDNF mRNA and protein expression in the hippocampus than controls, but higher levels of miR-16, which had a negative association with the expression of BDNF (Bai et al., 2012). Although there were several behavioral differences between MS rats and those subjected to chronic stress at 10 weeks, these did not affect BDNF expression in the chronically stressed group. MS rats showed more anhedonia and loss of interest, while chronically stressed rats showed more anxiety or depression-like behaviors.

Rats with 'abusive' mothers have been shown to have decreased levels of BDNF in the frontal cortex, and increased *BDNF* methylation at exon IV (Roth et al., 2009). This lasted into adulthood, and was reversed with administration of the methylation inhibitor zebularine. Maltreatment by a maternal figure results in widespread changes in gene expression, and is dependent on the sex of the offspring (Blaze and Roth, 2013). These results parallel observations in human adult PTSD patients, which demonstrate that those patients with a history of child abuse show markedly different DNA methylation levels and almost mutually exclusive patterns of gene expression (98% dissimilar) from those with no such history (Mehta et al., 2013).

Human studies of developmental stress

In humans, developmental stress has a clear impact on mental health in later life, e.g. (Edwards et al., 2003), however the biological mechanisms are still not entirely clarified. Nonetheless, epigenetic factors clearly play a role in susceptibility to stress-related disorders as the

work of Yehuda and others has made clear. More recently, compelling evidence for a lasting molecular epigenetic impact of early life trauma and adversity has rapidly accumulated (Radley et al., 2011; Klengel et al., 2013; McGowan, 2013; Mehta et al., 2013; Yehuda et al., 2014).

Recent work in this vein was published by Mehta and collaborators suggesting that a history of abuse may constitute a biologically distinct subset of PTSD patients from those without such a history. Individuals diagnosed with PTSD who have a history of childhood trauma had different levels of expression for 303 RNA transcripts compared to those who had some degree of trauma but had not been diagnosed with PTSD (Mehta et al., 2013). Individuals diagnosed with PTSD without a history of childhood trauma had 244 differentially expressed transcription profiles. Remarkably, only 14 transcripts overlapped between the two groups, signifying two distinct biological signatures for individuals diagnosed with PTSD, depending on their history of trauma. In individuals with PTSD and a history of child abuse, 69.3% of the genes for the differentially expressed transcripts had at least one CpG methylated site, and individuals with PTSD but no history of child abuse showed CpG methylation in 33.6% of genes that had been tied to differentially expressed transcription profiles. For genes with five or more methylated CpG sites, the gap widens to 11.7 and 0.8% for each group, respectively. It appears as if the differences in transcriptional profiles are mainly due to hypermethylation of DNA as a consequence of childhood trauma (Mehta et al., 2013).

Hypermethylation of the GR promoter was found in suicide victims with prior childhood abuse (McGowan et al., 2009; Labonte et al., 2012a,b). Further, individuals with depression but no history of child abuse did not show the same GR promoter methylation (Alt et al., 2010; Labonte et al., 2012a,b). The severity of traumatic events experienced in childhood had a direct relationship with the methylation of GR in adulthood (Perroud et al., 2014).

Child abuse may also result in the up-regulation of stress-regulatory genes. The AVP promoter and exons are hypomethylated, leading to decreased binding of the inhibitory CpG-binding protein MeCP2 and an increase in HPA activity (Murgatroyd et al., 2009). MeCP2 has also been linked to Rett syndrome (Kriaucionis and Bird, 2003) and BDNF expression (Chen et al., 2003; Martinowich et al., 2003).

Prenatal stress in animal models

Epigenetic programming is not just limited to post-natal life, but can begin while still in the womb (Howerton and Bale, 2012). Indeed, the epigenetic influence of stress can exert effects through multiple generations, as has been shown by the Meaney group in rodents (Francis et al., 1999). Prenatal stress follows a similar pattern to newborn stress: too much leads to structural and behavioral detriments. In response to stress, rat pups of stressed mothers showed a decrease in PVN volume, increased CRF mRNA expression (Fujioka et al., 1999), decreased hippocampal neurogenesis and worse outcomes on memory tasks through all stages of life (Lemaire et al., 2000). Conversely, rat mothers exposed to mild restraint

stress had pups that were less fearful, learned faster, and had lower c-Fos expression in the amygdala (Fujioka et al., 2001).

Mouse fetuses of stressed mothers were shown to have altered methylation of 11 β -hydroxysteroid dehydrogenase type 2, a corticosterone metabolite (Jensen Pena et al., 2012), and heightened levels of DNMT1/3a in GABAergic neurons. Prenatal stress in male rats produced behavioral deficits and decreased CRF promoter methylation, increased GR exon 1–7 methylation in the hypothalamus and reduced amygdala CRF promoter methylation (Mueller and Bale, 2008). Interestingly, these changes were not observed in females, and the sex-differentiated methylation corresponds to differential placental expression of several genes, such as DNMT1. Prenatal stress also alters methylation and expression of the structural protein GPM6A (Monteleone et al., 2013). MiR-133b showed increased levels of expression in the hippocampus, and cultures overexpressing miR-133b showed a decrease in GPM6A mRNA and function.

Prenatal stress in humans

The transgenerational effects of stress have also been observed in humans as a consequence of the dislocations and atrocities of the Second World War (Painter et al., 2008). Heightened levels of DNMT1/3a in GABAergic neurons in human psychiatric patients with psychotic symptoms were comparable to results found in prenatally stressed mice (Matrisciano et al., 2013). Increased HPA activation was also associated with increased methylation of the GR exon 1–7 homolog (1F) in human babies exposed to prenatal stress (Oberlander et al., 2008).

Critical periods

For both prenatal and neonatal stress, the most important factor may be the timing of critical periods (Callaghan et al., 2013). These are set time periods in which stress causes life-long epigenetic changes and behavioral deficits that may be difficult to overcome, despite how enriching their environment may be later. Critical periods have been shown to be important in miRNA expression (Morgan and Bale, 2011), and the methylation of DNA (Simmons et al., 2012, 2013). If prenatal stress occurs in the right critical period, epigenetic changes in the male germline can propagate and spread to the F2 generation via miRNA expression.

Three non-coding RNAs, miR-322, miR-574–3p, and miR-873, had higher levels of expression in the males of the F2 generation (Morgan and Bale, 2011). These three miRNAs all target the gene for β -glycan, whose function in the context of stress and plasticity is not completely understood. Another study using a model of combined unpredictable maternal stress and maternal separation found that the F2 generation showed more anxious behaviors than controls (Gapp et al., 2014). This coincided with the up-regulation of several additional miRNAs in the F1 generation's sperm: miR-375–3p, miR-375–5p, miR-200b–3p, miR-672–5p and miR-466–5p. Interestingly, the

F3 generation shows similar behavior as the F2 generation, despite the lack of miRNA upregulation in the F2 generation's sperm. Thus the precise mechanisms for this and other transgenerational effects require further study.

ACUTE STRESS

Our brains are relatively well-equipped to handle short-term stress, and normally show few negative aftereffects, yet even transient stresses can leave a lasting imprint. Fear conditioning is a robust example of this, often being acquired in one trial. It can result in drastic and long-lasting epigenetic changes in several brain areas including the amygdala (Monsey et al., 2011), hippocampus (Mizuno et al., 2012), and the mesolimbic dopaminergic system (Pezze and Feldon, 2004). Several epigenetic regulators have previously been demonstrated to change in response to fear conditioning, such as methyltransferase expression (Miller and Sweatt, 2007). Though most exposure to fearful situations is quickly resolved, some individuals continue to relive their traumatic experiences and suffer from PTSD (Yehuda and McFarlane, 1995). Because of this discordance, PTSD is of particular interest to the field of epigenetics because it has a clear cause-and-effect relationship with stress (McEwen et al., 2012; Zovkic and Sweatt, 2013).

PTSD in humans

Epigenetic changes in PTSD have been found at a number of loci. For example, higher methylation of the dopamine transporter allele 9r has been associated with higher incidences of PTSD in human participants (Chang et al., 2012). In a genome-wide methylation study, participants with PTSD had different levels of methylation at 119 CpG sites depending on socioeconomic status, with 55 sites differing with symptom severity (Uddin et al., 2013). Further analysis showed that the top interaction differences were involved in synaptic transmission and neuron projection pathways.

Another study examined veterans with PTSD and investigated the methylation levels of the GR gene in response to psychotherapy and found differences in GR methylation between those who responded to treatment and those who did not (Yehuda et al., 2013). The GR promoter region and GR exon 1F were hypermethylated before treatment in people who later responded to treatment. Furthermore, pre-treatment GR exon 1F methylation was correlated with symptom severity and the predictive of the amelioration of symptoms post-treatment. Those who responded positively to the treatment tended to be younger, had the disorder for less time, and had fewer traumatic events over the course of their lives. After psychotherapy, the levels of GR methylation did not change. FKBP5, a regulator of GR binding and translocation into the nucleus, had lower expression after treatment in those who responded to therapy.

FKBP5 has several functional mutations (Binder et al., 2004). Risk alleles are thought to decrease the functional abilities of GRs in an ultra-short feedback loop, and

carriers of one or more risk allele and exposure to childhood trauma have an increased risk of PTSD. Carriers of the risk alleles with no history of child abuse had decreased diagnoses of PTSD. Risk alleles of *FKBP5* were hypomethylated compared to protective alleles, with heterozygous individuals resembling risk-allele homozygous individuals. This points to demethylation as the putative source of GR dysregulation in the risk-allele carriers. The demethylation occurs in GC response elements of intron 7. Demethylation was also observed in hippocampal neuronal cultures exposed to dexamethasone. Pathways affected by the dysregulation of GRs and FKBP5 include T-cell receptor signaling, TGF- β signaling, Wnt signaling and pluripotency, and inflammatory response pathways (Klengel et al., 2013). Veterans who responded better to PTSD psychotherapy showed hypomethylation of the *FKBP5* promoter (Yehuda et al., 2013), though the researchers did not look at different alleles. The same study found that FKBP5 expression was positively correlated with cortisol levels.

Rusiecki et al. (2012) examined the global methylation of repeating elements in veterans. Those who were diagnosed with PTSD after deployment had stable levels of LINE-1 methylation, whereas those that did not develop PTSD had higher levels. Before deployment, veterans who would later develop PTSD had higher levels of Alu methylation. These findings suggest a difference in the global methylation of DNA, and a possible biomarker of people who might develop, or are currently suffering from, combat-related PTSD.

Stress has been shown to increase antisocial, and decrease prosocial, behaviors in part through the regulation of the oxytocin system (Olf et al., 2013). Understanding the epigenetic regulation of oxytocin represents a significant step toward elucidating the negative social effects of stress. The oxytocin receptor (*OXTR*) is encoded by a single gene (Zingg and Laporte, 2003). Expression of *OXTR* is influenced by two transcription factors, ER α and SP1 (Safe and Kim, 2008). The *OXTR* promoter region has several CpG sites, two of which correspond with ER α and SP1 binding sites (1 and 7, respectively), and methylation of CpG 1 or 7 is tissue specific and influences the expression of *OXTR* in different brain regions (Mamrut et al., 2013). Despite sex-specific roles of oxytocin, *OXTR* methylation is sex-independent. *OXTR* is differentially methylated in several brain areas, including the ventromedial hypothalamus (Harony-Nicolas et al., 2014), which has been shown to have inhibitory effects on the HPA pathway (Suemaru et al., 1995).

Healthy human adults subjected to the Trier social stress test showed differentially methylated *OXTR* in blood samples (Unternaehrer et al., 2012) collected pre-test, 10 min post-test, and at 90 min. Post-test samples showed higher levels of methylated CpG sites associated with one region of the gene, while the 90-min point showed a large decrease in methylation at both sites examined. The extent to which peripheral measures such as these correlate to central phenomena is a common question in studies of this sort. However, these results suggest that the genes responsible for the regulation of

affiliative behavior are under dynamic epigenetic control in response to social stress.

Early epigenetic investigations revealed that a number of psychiatric drugs in common use, such as valproate, clozapine and imipramine had epigenetic activities (Boks et al., 2012). As a consequence, many researchers are exploring the use of HDAC inhibitors and other epigenetically active drugs to increase acetylation of histones with the goal of understanding its effect in stress-related disorders. Human participants given the HDAC inhibitor valproic acid (VPA) had lower skin conductance (SC) responses in response to conditioned fear after sleep, and when given an hour and a half before testing, VPA had an anxiolytic effect (Kuriyama et al., 2013). VPA administration before trials also decreased learning acquisition and increased extinction on a SC response compared VPA administration directly after trials. Participants who were given VPA before sleep had lower SC responses than those who were given VPA and stayed awake and the control sleep group. Conversely, participants given α -cycloserine (DCS), a partial N-methyl-D-aspartate receptor (NMDAR) agonist, had higher SC responses in the awake group compared to the sleep group, and higher than the waking controls, but DCS had no effect on learning and extinction trials. NMDAR blockade has previously been shown to prevent memory formation and BDNF expression (Lubin et al., 2008).

A valine (Val) to methionine (Met) single nucleotide polymorphism (SNP) in the gene that encodes catechol-O-methyltransferase (COMT), which removes dopamine from synapses that lack dopamine transporters (Chen et al., 2004), resulted in differentially methylated COMT gene (Norrholm et al., 2013). This SNP resulted in behavior deficits in the inhibition of fear stimuli. Individuals with PTSD and homozygous Met/Met responded more fearfully to “safe” stimuli than individuals with Val/Met or Val/Val genotypes. They also responded more fearfully to “safe” stimuli than individuals who were not diagnosed with PTSD and shared the Met/Met genotype. Participants who were more fearful also have higher levels of COMT DNA methylation.

Animal models of acute stress

Animal models have contributed substantially to our understanding of both molecular and behavioral epigenetics and acutely stressful manipulations have shown a number of such effects on the brain and behavior. Many of these studies have used psychiatric drugs with known interactions with epigenetic writer or eraser enzymes, however, given that many of these drugs have well described interactions with neurotransmitter systems, for example, interpreting their effects in an exclusively epigenetic light not always tenable. BDNF is a target of GR transactivation and differentially expressed in response to stress (Bennett and Lagopoulos, 2014). Histone H4 acetylation and its influence on BDNF expression may contribute to the extinction of fear memory associated with VPA administration before sleep (Bredy et al., 2007). Female mice exhibited increased methylation of BDNF in response to fear,

and activating the expression of BDNF blocked the return of fear memory (Baker-Andresen et al., 2013).

Similar results were seen in rats treated with vorinostat, another HDAC inhibitor. In combination with extinction training, the rats had reduced freezing times, increased H3 and H4 acetylation, and changes in NMDAR subunit prevalence (Matsumoto et al., 2013). Rats in the stress and vorinostat group had increased levels of the NMDAR sub-unit, NR2B, mRNA and associated protein (but not NR1 or NR2A) in the hippocampus compared to just the vorinostat group. Stress and vorinostat also increased the levels of CaMKII α and CaMKII β proteins compared to the stress and vehicle group. Upregulation of NR2B has been previously shown to be associated with fear extinction (Tang et al., 1999), and CaMKII is known to cooperate with NMDARs.

Rats given a high dose (1200 mg/kg) of the HDAC inhibitor sodium butyrate (NaBu) had increased plasma ACTH, corticosterone, and glucose levels when measured 60 min after administration compared to controls (Gagliano et al., 2013). To test NaBu's dose effect, the researchers administered a low dose of NaBu (200 mg/kg), high dose of NaBu (1200 mg/kg), and a hypertonic saline solution (HT). Only the high-dose group showed increased plasma levels of ACTH, corticosterone, and glucose 120 min post-injection compared to a low dose and the HT group. C-Fos expression was elevated for the high dose of NaBu compared to controls, but did not differ from the HT group in the central amygdala and supraoptic nucleus. NaBu did, however, result in elevated c-Fos expression above the HT group in the PVN and the ventral lateral septum, pointing to these brain areas as putative sites for NaBu's influence on the stress response.

As one might anticipate, HAT inhibition produces cognitive and behavioral effects generally opposite of those produced by HDAC inhibition. Garcinol is a naturally occurring HAT inhibitor derived from a species of mangosteen used in South Asian cuisine. Rats injected with garcinol in the lateral nucleus of the amygdala had lower levels of histone H3 acetylation, impaired long-term memory (LTM), and failed to consolidate previously learned material (Maddox et al., 2013). When injected 1 h after fear conditioning, rats showed no difference in freezing behavior. They did show impaired LTM a day later, but not when injected with garcinol 6 h after the fear-condition training. Likewise, rats subjected to fear conditioning the following day and then given an injection of garcinol showed impaired LTM of the recalled fear memory. As in the original condition, rats that received the injection and failed to consolidate memory also had lower levels of H3 acetylation.

Manipulating histone modifications through drugs is an exciting new field of research for those suffering from stress and anxiety disorders. While HATs and HDACs are not fully understood, especially since part of their mechanism of action may involve the acetylation of non-histone proteins, they have proven to be effective at altering learning and memory. HAT inhibitors seem to interfere with the consolidation or reconsolidation of memories, while HDAC inhibitors increase the production of neuroprotective proteins, and have even been shown

to reverse some effects of neuronal loss and recover some brain plasticity (Fischer et al., 2007). The *HDAC2* gene has been shown to regulate memory and plasticity through control of glutamate receptors and BDNF (Guan et al., 2009), and HDAC activity is required for BDNF functioning (Calfa et al., 2012).

NF- κ B is a master transcriptional regulator of inflammatory genes and implicated in depression (Miller et al., 2009). The NF- κ B pathway is highly regulated by stress, and different NF- κ B family members are differentially expressed in the brain as a consequence of acute and chronic stress (Gray et al., 2013a,b). *Nfkbia* showed increased transcription in male mice in response to a force swim test. Auditory fear memory was blocked from reconsolidation by I κ B kinase inhibitor (sulfasalazine) or NF- κ B inhibitor (SN50) administration in the basolateral amygdala (BLA), and was rescued by pretreatment with NaBu (Si et al., 2012). Rats spent less time freezing 24 h after intra-BLA sulfasalazine, but not after treatment of the central nucleus of the amygdala, and only when administered directly after re-exposure to the fear stimulus. When rats were treated with SN50 2 h before re-exposure, it had a similar effect. Both drugs reduced freezing behaviors through the presumed increased activation of NF- κ B. Treatment with the HDAC inhibitor NaBu restored memory retrieval and brought freezing behavior back up to the level seen in controls.

Two hours of restraint produced a substantial increase in corticosterone levels in rats, and resulted in the decreased expression of miR-135a and miR-124 in the amygdala (Mannironi et al., 2013), both of which had previously been shown to block the translation of the MR mRNA, though not its transcription (Söber et al., 2010). However, Mannironi et al. found that overexpression of miR-135a led to a marked decrease in the expression of the *Nr3c2* mRNA, which encodes the MR protein (Mannironi et al., 2013). Transfecting cells that did not endogenously express either miR-124 or miR-135a resulted in the decrease in MR levels. Transfected cells expressing either miR-124 or miR-135a had lower levels of MR protein, and perhaps more importantly, cells with both showed a further decrease beyond either one alone. Inhibiting the miRNAs resulted in the increased expression of MR proteins. In addition to MR inhibitory action, miR-124 has been shown to directly inhibit the translation of GR mRNA (Vreugdenhil et al., 2009).

Other miRNAs have been shown to be affected by the acute stress response. The GR mRNA is blocked from translation by miR-18a in rats and humans (Turner and Muller, 2005; Uchida et al., 2008). The amygdala is the target of several differentially expressed miRNAs in response to stress, including miR-34, miR-134, and miR-183 (Meerson et al., 2010; Haramati et al., 2011). Increased miR-132 expression in response to stress reduced levels of acetylcholinesterase (AChE) in rats (Shaltiel et al., 2013). Stress in mice and rats has also been shown to have an effect on several miRNAs in the hippocampus, frontal cortex, and cerebellum (Mongrain et al., 2010; Rinaldi et al., 2010; Babenko et al., 2012).

Transposable elements represent a new frontier for both epigenetics and genomics in the brain, and recent

work has shown that they are clearly regulated by stress. Acute stress also has impacts on TEs. Stress increases expression of the methyltransferase *Suv39h2*, increasing H3K9 trimethylation at tens of thousands of TEs in the hippocampus, in less than 2 h, consequently decreasing TE RNA expression (Hunter et al., 2012). In a model of PTSD, stress also results in the differential regulation of TEs in the amygdala (Ponomarev et al., 2010).

CHRONIC STRESS

Many stressors are temporary in nature; however, some are not. Chronic and acute stress affects both common and divergent pathways and mechanisms, including epigenetic ones such as differential HDAC activity (Renthal et al., 2007). When the organism is overtaxed through chronic stress, it can have deleterious effects on mood and behavior. Sometimes the stressful event is external, such as poverty, and sometimes it is a persistent internal state, such as major depression. Stable changes to the epigenome represent a likely mechanism for chronic stress disorders, and an interesting avenue for possible therapeutic intervention.

Animal models of chronic stress

Chronic social defeat (CSD) is a model of chronic stress and depression with high face and ethological validity (Nestler and Hyman, 2010), which results in widespread changes in gene expression, histone methylation, and phospho-CREB binding in the nucleus accumbens, all of which showed reversal through treatment with imipramine (Wilkinson et al., 2009). CSD increased repressive methylation of the *BDNF* promoter in the hippocampus, which was reversed by antidepressants (Tsankova et al., 2006). CSD has also been shown to regulate DNMT3a in the nucleus accumbens, and exogenous DNMT3a administration causes depressive-like behavior in rats (LaPlant et al., 2010). CSD decreased methylation of *CRF* in mice. *CRF* is not hypomethylated in resilient mice (Elliott et al., 2010). The epigenetic and behavioral changes that followed treatment with imipramine were similar to that in resilient mice (Wilkinson et al., 2009). Submaximal social defeat was not enough to produce the changes seen in CSD, unless accompanied by cocaine administration (Covington et al., 2011). Cocaine's action on H3K9me2 and G9a mirrored a local knockout of G9a, which showed behavioral similarity to CSD and depression in humans. G9a is also responsible for the antidepressant-caused inhibition of DNMT1 (Zimmermann et al., 2012).

Rats exposed to chronic variable stress spent less time exploring novel objects, had a corresponding reduction in extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) in the hippocampus (which leads to lower levels of downstream products such as the anti-apoptotic protein Bcl-2 and an increase in SIRT1, an HDAC) and a decrease in histone H4 acetylation. These changes are reversed by treatment with sirtinol, a SIRT1 inhibitor (Ferland et al., 2013). Rats exposed to chronic stress spent less time exploring a

novel item in their cage compared to non-stressed animals, and showed no preference for it over a previously seen object. This behavioral difference was matched with a reduction in ERK1/2 in the CA1, CA3, and dentate gyrus regions of the hippocampus. The reduction in Bcl-2 is associated with an increase in the acetylation of the *Bcl-2* promoter in the dentate gyrus, but not in other genes important for long-term memory. Previous research had shown an increase in SIRT1 activity in the dentate gyrus in response to chronic stress (Ferland and Schrader, 2011). The current study found an injection of sirtinol into the dentate gyrus did not affect the HPA stress response, but did result in decreased SIRT1 activity. Sirtinol caused a further decrease in SIRT1 activity of chronically stressed animals relative to sirtinol-treated controls. The injection of sirtinol reversed the decrease in H4 acetylation and the decrease in ERK1/2 levels seen in chronically stressed animals, and increased Bcl-2 expression. Sirtinol also rescued the behavioral deficits seen in novel object exploration and restored preference for sucrose. Overall, this points to SIRT1 activity as a mechanism of chronic stress-induced deficits in behavior, ERK1/2 activity, Bcl-2 expression, and the acetylation of histone H4.

Male rats subjected to 6 weeks of stress before being bred sired offspring with blunted stress responses (lower corticosterone levels) which could not be accounted for by changes in single gene expression, but did correspond in increased levels of nine miRNAs (Rodgers et al., 2013). Despite the lower levels of corticosterone in response to brief restraint, the offspring of stressed rats did not behave differently in a battery of behavioral tests compared to controls, nor did they have changes in gene expression of CRFr1, POMC, Mc2r, 11 β HSD-1. Treatment with SSRIs did not affect the offspring of paternally stressed rats differently than controls. Paternal stress enriched GSEA gene sets c3 and c5. Nine miRNAs had higher levels of expression in offspring of paternally stressed rats. These results were similar both in paternal rats stressed throughout puberty, or only in adulthood. Sperm do not have many of the epigenetic regulatory elements that other cells have, but do carry miRNAs that are transmitted during fertilization. Some miRNAs are necessary for successful development (Liu et al., 2012). Male mice who experienced MS also had changes in sperm DNA methylation (Franklin et al., 2010), although it is still unclear on how much effect this method of epigenetic transmission has on offspring. MiRNA also presents itself as a target of anti-depressants, and there is some evidence that electroconvulsive shock therapy influences expression of miRNAs (O'Connor et al., 2013).

A mouse model of psychotic depression with non-functioning *DISC1* alleles was exposed to isolation stress in adolescence for 3 weeks, and as an adult, showed a GR-controlled hypermethylation of the gene that encodes tyrosine hydroxylase, an important enzyme in the production of dopamine (Niwa et al., 2013).

Depression and suicide in humans

In depressed patients, *BDNF* promoter methylation was positively associated with suicide ideation and attempts.

Lower methylation of the *BDNF* promoter reflects poorer treatment outcomes (Kang et al., 2013). Previous studies have shown higher methylation of the *BDNF* promoter region in suicide completers (Keller et al., 2010). Chronic stress can result in both hyper- and hypomethylation of *BDNF* in different areas of the same brain structure (Roth et al., 2011), but generally lower *BDNF* methylation in blood samples is associated with depressive symptoms (Fuchikami et al., 2011).

CONCLUSION

The study of epigenetics has substantially enriched our understanding of the mechanisms by which the brain adapts to stress. Particularly striking are the numerous observations that some epigenetic changes caused by stressors and environmental insults may be passed on to subsequent generations (Skinner et al., 2008; Morgan and Bale, 2011; Crews et al., 2012), either through maternal care (Francis et al., 1999) or other epigenetic mechanisms (Guerrero-Bosagna et al., 2010; Rodgers et al., 2013). Paternal contributions to the development of a fetus are often overlooked, but recent findings of sperm-based epigenetic inheritance may provide insight into previously unexplained issues, e.g. (Dietz et al., 2011).

While transgenerational effects are remarkable for their persistence, epigenetic mechanisms also underlie some of our ability to flexibly adapt to and overcome stressful circumstances. Resilience to stress can be instilled through controlled and controllable exposure to stress (Russo et al., 2012). For example, rats exposed to levels of prenatal stress that would normally result in behavioral deficits later in life can have those deficits rescued by postnatal handling (Lemaire et al., 2006).

We have chosen to organize this review on a temporal basis between acute and chronic stressors as the study has long made clear that the two types of stress have distinct and often opposed effects on the organism. It is apparent that the epigenetic impacts of stress differ between acute and chronic manipulations, often producing opposing effects, and this seems to be the case for the effects of some drugs of abuse as well (Nestler, 2014). Indeed, recent work from our group has clearly shown that there is very little overlap in the changes in gene expression produced by acute and chronic stress in the rodent hippocampus, as the two conditions overlap by less than 4% (Gray et al., 2013a,b). This suggests, given the impact of epigenetic marks on transcriptional landscapes, that the distinction between acute and chronic exposures is born out in epigenetics as it has been in other aspects of stress biology. Of course, these effects, though opposed in direction may involve differing levels of the same mark and global levels of a particular mark are likely not as informative as the overall architecture of interaction between epigenetic modifications, transcription factors etc. which are highly computationally complex and as yet poorly understood.

The extent to which this adaptive flexibility can be enhanced with epigenetically targeted pharmacotherapies in concert with structured behavioral interventions such as cognitive behavioral therapy remains to be seen, but

the fact that so many of the drugs in common psychiatric use have epigenetic activity (Boks et al., 2012) suggests that an answer could be extracted via meta-analysis of existing clinical data sets, though these will not be adequate to determine mechanism. It is worth noting that the discovery of the epigenetic activity of a number of psychiatric drugs only adds to the ambiguities in our understanding of their actions. An example of this might be any of a number of antidepressants, which, despite their immediate action on what are thought to be their clinically important molecular targets (e.g. the serotonin transporter), still take weeks to act. Much mechanistic pharmacology remains to be done before epigenetic pharmacology can be put on a firm footing, yet it is clear that the field is worthy of the effort.

Much systematic work remains to be done to understand the complex relations between the genome, environment and epigenetic factors can be fully appreciated in the context of the neurobiology of stress, but the past decade of work has clearly established the importance of these mechanisms to our understanding of the impact of stress in disease and heredity.

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