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Review

Epigenetics of Stress-Related Psychiatric Disorders and Gene × Environment Interactions

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A deeper understanding of the pathomechanisms leading to stress-related psychiatric disorders is important for the development of more efficient preventive and therapeutic strategies. Epidemiological studies indicate a combined contribution of genetic and environmental factors in the risk for disease. The environment, particularly early life severe stress or trauma, can lead to lifelong molecular changes in the form of epigenetic modifications that can set the organism off on trajectories to health or disease. Epigenetic modifications are capable of shaping and storing the molecular response of a cell to its environment as a function of genetic predisposition. This provides a potential mechanism for gene-environment interactions. Here, we review epigenetic mechanisms associated with the response to stress and trauma exposure and the development of stress-related psychiatric disorders. We also look at how they may contribute to our understanding of the combined effects of genetic and environmental factors in shaping disease risk.

Introduction

Psychiatric disorders and in particular stress-related psychiatric disorders such as post-traumatic stress disorder (PTSD), major depressive disorder (MDD), and anxiety disorders are multifactorial diseases influenced by both genetic predisposition and environmental factors (Stein et al., 2002; Sullivan et al., 2000). Adverse life events, especially early in life, have consistently been shown to strongly increase the risk for mood and anxiety disorders in large epidemiological studies (Kessler et al., 1997). Although severe forms of early adverse life events such as childhood abuse or neglect have been associated with the highest rates of increased risk (Dube et al., 2001), other forms of early adverse experiences, such as parental loss, bullying, or low socioeconomic status in childhood, were also shown to consistently increase risk for a number of psychiatric disorders (Kessler et al., 2010). Finally, an increasing body of literature suggests that prenatal adversity, in the form of stress or mood and anxiety disorders of the mother, is also a risk factor for psychiatric disorders (Stein et al., 2014). A factor common to these early adversities is that they have all been associated with long-term changes in the regulation of the stress hormone system (Lupien et al., 2009), as illustrated in Figure 1, which may be causally related to the development of disease. In addition to the strong effects of the environment, there is a significant genetic contribution to the development of these disorders (Kendler et al., 2006; Sullivan et al., 2000). However, strong main genetic effects have not been observed for stress-related psychiatric disorders to date, reflected by a lack of genome-wide significant associations in studies with sample sizes that have led to robust genetic association signals for schizophrenia and bipolar disorder (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014; Ripke et al., 2013; Mühleisen et al., 2014). The genetics of stress-related disorders are therefore confronted with the so-called missing heritability that describes the lack of strong effects in the kind of gene-association studies found in twin and family studies (Lee et al., 2013). This is likely accounted for by weak phenotype definitions potentially leading to a dilution of genetic effects. Current diagnostic classification includes a number of pathophysiological subtypes under the broad definitions of anxiety and depressive disorders. In addition, genetic factors may have considerably smaller effect sizes compared to schizophrenia where the explained variance by polygenic factors has consistently increased with growing sample sizes (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). In MDD, anxiety disorders, and PTSD, the reliable detection of such polygenic risk factors may need much larger samples (Levinson et al., 2014).

Environmental factors as major triggers of stress-related disorders may lead to additional heterogeneity that is unaccounted for in current genetic studies. Understanding the molecular embedding of risk, conferred by adverse life events and how these interact with genetic vulnerability, may be important for the identification of the missing heritability observed in stress disorders. One molecular mechanism that has come into focus for mediating long-term environmental effects is epigenetics (Slatkin, 2009).

Epigenetics subsumes mechanisms of functional control over the genetic information without changing DNA sequence. These mechanisms include the post-translational modification of histone proteins as well as chemical modifications of single nucleotides (most commonly in the form of DNA methylation or hydroxymethylation at cytosine residues), which alter the chromatin structure and thus the accessibility of the DNA to transcriptional regulators. In the broader sense of epigenetic regulation, these mechanisms also include the regulation of transcription and translation by non-coding RNAs, as schematically represented in Figure 2. We here intentionally include regulation by non-coding RNAs because of their ability to regulate transcriptional and translational output in post-mitotic neurons and direct epigenetic modifiers to specific loci (Bird, 2007; Bonasio

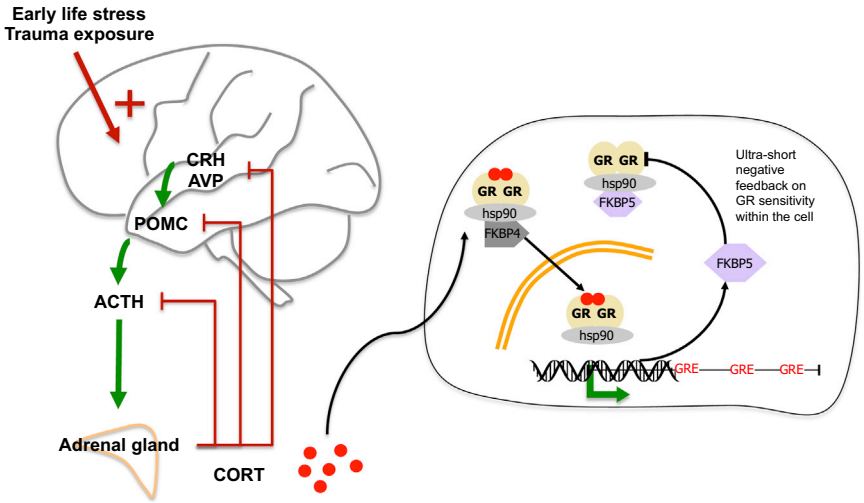

Figure 1. Stress and, in Particular, Early Life Adversities Activate the Stress Hormone System and May Epigenetically Program the System toward a Lifelong Alteration of the Hormonal Response to Even Minor Stressors

The neuropeptides corticotrophin-releasing hormone (CRH) and vasopressin (AVP), released from the hypothalamus in response to stress, activate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, finally leading to an increased systemic cortisol secretion from the adrenal gland. Cortisol binds to steroid receptors, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR), that act as transcriptional activators or repressors in the nucleus through binding to glucocorticoid response elements. This influences the expression of numerous genes involved in the stress response, immune function, and metabolism. Binding of the GR and transcriptional activation of, for example, FKBP5 provide an ultrashort feedback to the GR, terminating the stress response and secretion of cortisol.

et al., 2010; Egger et al., 2004; Holliday, 2006; Jaenisch and Bird, 2003; Jenuwein and Allis, 2001; Peschansky and Wahlestedt, 2014). Some epigenetic modifications, especially DNA methylation, have been considered irreversible in the past, defining cellular identity in a multicellular organism. By now it has been shown that even stable chemical modifications, such as DNA methylation, underlie highly dynamic regulation. This potential reversibility makes these mechanisms suitable for encoding the long-term impact of the environment also in post-mitotic tissue such as neurons (Sweatt, 2013). Although often depicted separately for clarity, epigenetic mechanisms form a complex interactive network with joint activities of different mechanisms contributing to the same transcriptional regulation.

The field of epigenetics thus provides a possible molecular framework of how genetic and environmental factors interact and shape the risk for psychiatric disorders (Sweatt, 2013). Epigenetics has been shown to play a decisive role in the neuronal adaptations underlying learning and memory (Zovkic and Sweatt, 2013), the response to environmental challenges (Champagne, 2010; Jirtle and Skinner, 2007; McGowan and Szyf, 2010; Zhang and Meaney, 2010) and the pathogenesis of mental disorders (Bale et al., 2010; Jakovcevski and Akbarian, 2012; Mill and Petronis, 2007; Tsankova et al., 2007; Vialou et al., 2013).

We here review the current knowledge on epigenetic modifications in response to environmental factors and their interaction with genetic predisposition for stress-related diseases. In particular, we focus on the effects of childhood abuse and neglect, the environmental factors conveying the most consistent increase in risk, the epigenetic changes, and how they may relate to the development of stress-related psychiatric disorders. We will be discussing the current evidence of epigenetic mechanisms, in particular DNA methylation, as a potential molecular link between environmental exposures and risk for psychiatric disorder, as the vast majority of human studies have investigated this modification with corresponding studies in laboratory animals, and discuss how they can contribute to gene by environment interactions (G×E).

Modification of Epigenetic Profiles by Severe Stress and Trauma in Early Life—Potential Mechanisms

In addition to a growing number of animal studies indicating long-lasting epigenetic effects of early stressful environments, a number of studies in humans now also suggest that such mechanisms may play a role in stress-related psychiatric disorders. In contrast to animal studies that can focus on brain tissue, most human studies have been performed in mixed tissues that are accessible to molecular investigation, such as peripheral blood and buccal cells, with only few studies investigating post-mortem brain tissue. Initial studies followed hypothesis-driven, candidate-based approaches, but recent advances in array- and sequencing-based techniques allowed the interrogation of epigenetic marks on a genome-wide level as recently reviewed in Klengel et al. (2014). Among the very first candidates implicated in stress-related epigenetic regulation were genes involved in the stress- or hypothalamus-pituitary-adrenal (HPA) axis due to its prominent role in the pathophysiology of stress-related disorders. Other candidate gene-driven studies were led by initial findings from genetic and gene expression studies investigating epigenetic modifications in genes involved in monoaminergic or neurotrophic signaling. However, unbiased, genome-wide studies have implicated epigenetic changes in genes often unrelated to established candidates, implicating alternative pathophysiological mechanisms. These studies suggest that epigenetic mechanisms are important in stress-related disease, but they remain on a descriptive, associative level. Largely due to the relative unavailability of human brain tissue, very little is known about how these differences may be established and maintained into adulthood and how they could lead to psychopathology. Furthermore, it remains unclear to what extent changes in peripheral tissues reflect changes in the CNS and which molecular processes may be shared across tissues. In the following paragraphs we highlight the potential mechanisms known to date by which early life stress may lead to a permanent imprint of the stressor onto the genome by epigenetic modification, relying predominantly on non-human literature, and these are summarized in Figure 3. We later focus

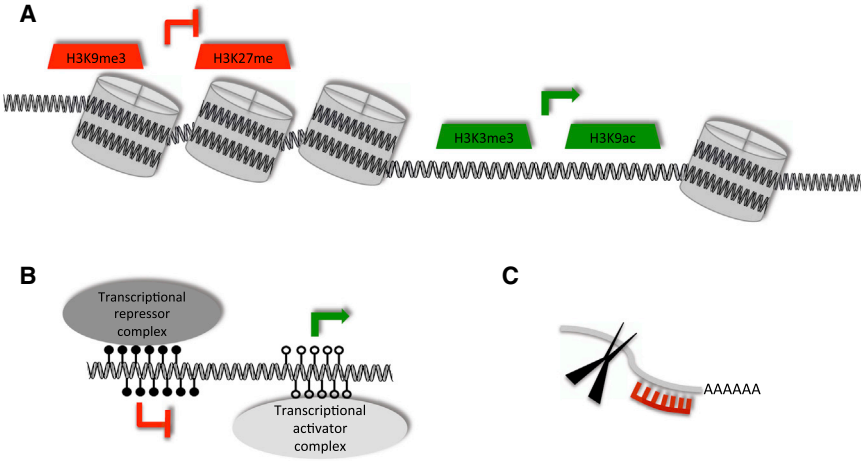

Figure 2. Schematic Representation of Main Features of Epigenetic Regulation by Post-translational Histone Modification, DNA Methylation, and Non-coding RNA

This overview explicitly reduces and simplifies the complex and multifaceted mechanisms of epigenetic regulation for clarity. More specialized reviews for a deeper description of this matter are given in the text.

(A) Histone modifications influence the condensation of the DNA around histone proteins and regulate the accessibility of functional regions to transcriptional regulators, through modification at predominantly the N-terminal tails, altering the spatial structure of the chromatin and the interaction with DNA-binding proteins. Contingent on the location and the type of modification, this can lead to a more condensed chromatin-repressing active transcription (exemplified by histone H3, lysine 27 dimethylation (H3K27me2) and histone H3, lysine 9 trimethylation (H3K9me3)) or vice versa to an open chromatin state facilitating active transcription (exemplified by histone H3, lysine 4 trimethylation (H3K4me3) and histone H3, lysine 9 acetylation (H3K9ac)).

(B) DNA methylation predominantly at CG dinucleotides (CpG) can influence the spatial structure of the DNA and the binding of or repression of specific DNA-binding proteins to the DNA. The closed circles represent higher methylation at cytosine residues, and the open circles represent lower methylation. Methylation around the transcription start site in the promoter and the first exon is usually accompanied by transcriptional silencing. DNA methylation at other regulatory regions and in the gene body can also facilitate transcription. Not depicted here are other modifications such as hydroxymethylation.

(C) Non-coding RNAs that include, for example, miRNA can influence chromatin structure and protein binding to the DNA but also directly target transcription and translation. Depicted here is the regulation of mRNA stability through binding of miRNAs at the 3′UTR of target mRNA that can lead to a decrease in mRNA stability, a decrease in mRNA cleavage, and therefore a reduction in protein assembly.

on human epigenetic studies and how they shape our understanding of the development and treatment of psychiatric disorders.

Neuronal Activation Leading to Post-translational Modifications of Epigenetic Readers and Writers

The post-translational modification of epigenetic readers and writers is an example of how stress can exert a long-term impact on gene regulation. An example is methyl CpG binding protein 2 (MeCP2) that influences transcription in response to neuronal activation. MeCP2 directly binds to methylated DNA, acting as transcriptional repressor, but it has also been shown to interact with other proteins such as cAMP response element-binding protein (CREB) and in this conformation can trigger transcriptional activation as well (Chahrour et al., 2008). An activity-dependent modification of MeCP2 appears to be involved in the long-term de-repression of the arginine vasopressin (*avp*) gene activity in response to maternal separation in mice. Murgatroyd et al. showed that maternal separation leads to the phosphorylation of MeCP2 and thus to the dissociation from the promoter region of the murine *avp* gene in the paraventricular nucleus. Subsequently, the MeCP2 binding site is demethylated, leading to a sustained transcriptional activation of the *avp* gene by reduced binding of the MeCP2 repressor complex (Figure 3A) (Murgatroyd et al., 2009). The early priming to demethylation by early life stress (ELS) exposure may be mediated by polycomb complexes and ten-eleven translocation (TET) proteins that attract DNA methyl transferases and histone deacetylases to ensure proper methylation status of the locus. The binding of these proteins is reduced following stress-dependent phosphorylation and dissociation of MeCP2 (Murgatroyd and Spengler, 2014). However, the exact signaling cascade from the maternal separation to locus- and cell-type-specific DNA demethylation remains unknown.

MeCP2 also targets other genes implicated in the regulation of the HPA axis. Nuber et al. (2005) showed that MeCP2 knockout mice have elevated mRNA expression levels of FK506 binding protein 5 gene (*fkbp5*), serum and glucocorticoid-regulated kinase 1 (*sgk1*), and other glucocorticoid-responsive genes without substantially increased glucocorticoid plasma levels, providing evidence that MeCP2 can function as a modulator of glucocorticoid action in neuronal cells (Nuber et al., 2005). More recent studies show that MeCP2 interacts with a plethora of other chromatin-modifying enzymes depending on the phosphorylation status of the protein, which in turn leads to a directed modulation of transcription of a given locus (Bellini et al., 2014; Ebert et al., 2013). Stress-related post-translational changes of this protein may thus have wide-ranging epigenetic effects.

Direct Transcriptional Regulation of Epigenetic Writers, Readers, and Erasers by Stress

Stress can also directly influence the transcriptional regulation of epigenetic writers, readers, and erasers. This has been shown for the transcriptional repression of DNA (cytosine-5)-methyltransferase 1 (DNMT1), the main enzyme responsible for the maintenance of DNA methylation, by glucocorticoid exposure as a proxy for stress. Lee et al. demonstrated that in vitro exposure of a murine pituitary cell line but also in vivo exposure of mice to the glucocorticoid analog dexamethasone correlated with a dose-dependent decrease in *dnmt1* expression and reduced DNA methylation at the murine *fkbp5* locus (Figure 3B) (Lee et al., 2011). However, whether the demethylation of the *fkbp5* locus is directly connected to this reduction remains an open question. In adult animals, stress has been shown to regulate *dnmt* expression in a brain-regions-specific manner (Elliott et al., 2010; LaPlant et al., 2010).

Other studies have shown that early life stress can influence the expression of histone deacetylases (HDACs)—enzymes

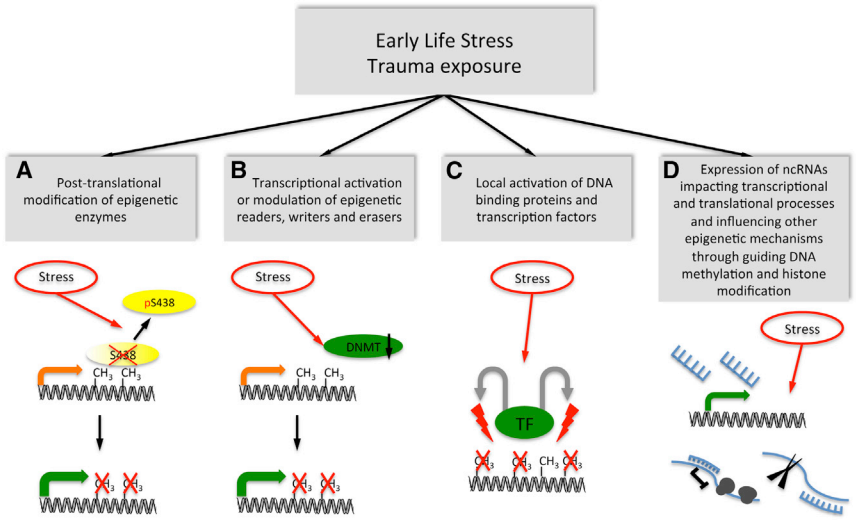

Figure 3. Stress May Influence the Epigenome on Different Levels and via Distinct Mechanisms

(A) As shown for maternal separation, early life stress impacts the post-translational modification of the epigenetic modifier MeCP2 by phosphorylation, leading to a dissociation of the protein complex from the DNA strand. Subsequently, DNA is demethylated, influencing the ability of proteins to bind to the DNA and repress transcription.

(B) Stress can also change the transcriptional activity of epigenetic enzymes, thus leading to an increased or decreased presence of the resulting proteins and subsequent alterations of epigenetic profiles.

(C) Binding of DNA-binding proteins such as transcription factors may change the underlying local epigenetic pattern by itself.

(D) Stress also influences the expression of noncoding RNAs, such as miRNA, that in turn can influence mRNA stability and translation of many genes in a signaling pathway.

that lead to more condensed chromatin structure—in the murine brain (Levine et al., 2012; Tesone-Coelho et al., 2015). Suri et al. suggested an age-dependent expression of histone-modifying enzymes in response to early life stress with a decreased expression in young animals and an increased expression in adult mice when exposed to early life stress (Suri et al., 2014). This opposite expression of HDACs over time was consistent with distinct global mRNA expression profiles separating young from adult animals exposed to early life stress. Likewise, Blaze and Roth observed that adverse rearing conditions only led to minimal differences in the expression of DNMTs, HDAC1, and MeCP2 in the medial prefrontal cortex of developing rats but found that these changes increased and became significant in adulthood in a sex-specific manner (Blaze and Roth, 2013). Numerous studies investigating the acute but also long-term consequences of stress in rodents now show the importance of epigenetic writers, readers, and erasers in establishing and maintaining specific marks in response to stress, as reviewed by Peña et al. (2014). These findings in animals are paralleled by less extensive human studies. Sipahi et al., for example, investigated the longitudinal DNA methylation profile of the genes encoding DNMTs pre- and post-trauma exposure in peripheral blood cells. They found that *dnmt1* methylation increases after trauma exposure only in individuals who went on to develop PTSD, while *dnmt3a* and *dnmt3b* methylation, the enzyme subtypes mainly responsible for the establishment of genomic DNA methylation pattern, increase in trauma-exposed individuals regardless of PTSD status, thus differentiating PTSD-susceptible and -resilient trauma-exposed individuals (Sipahi et al., 2014). Overall, these data suggest that stress may induce long-lasting epigenetic changes by altering the expression of genes critically involved in epigenetic regulation. For now, it is not clear, however, whether the expression changes that were all observed in mixed tissues actually only apply to very distinct cell types or are more global. How changes in the levels of DNMTs or HDACs can actually lead to epigenetic modifications at specific loci also requires further investigations, but interaction with other proteins and transcription factors guiding these epigenetic proteins is a plausible explanation (Hervouet et al., 2009; Joshi et al., 2013).

Activation of Transcription Factors that Lead to Local Changes in the Epigenetic Profile

An additional molecular mechanism leading to long-term epigenetic changes in response to stress is the activation of specific transcription factors that in turn lead to local changes in epigenetic profiles. Early reports on the transcription factor Sp-1 showed that binding of Sp-1 leads to a local inhibition of de novo DNA methylation (Brandeis et al., 1994). Furthermore, glucocorticoid receptor (GR) activation can lead to a local demethylation of GR response elements (GREs) (Thomassin et al., 2001). The mechanism of GR-induced local demethylation has not been fully understood, but the DNA repair machinery was implicated in this process, allowing the replacement of methylated by unmethylated cytosines. This demethylation of GREs subsequently facilitates the transcriptional effects of the GR on the target gene (Figure 3C) (Kress et al., 2001, 2006). Another example is the activation of the Nuclear Factor 1 A-type (NF1A) transcription factor by maternal care in rodents. Weaver et al. showed that high levels of maternal care in early life are linked to serotonin signaling in the rat hippocampus with an increase in expression of the transcription factor nerve growth factor-induced protein A (NGFI-A). This is the transcription factor that binds to the I_7 promoter of the rat GR gene, increasing its expression. Binding of NGFI-A leads to a decrease in methylation of the promoter with subsequent higher transcription factor binding and increased GR expression (Weaver et al., 2004; Zhang et al., 2013). Recently, collaborative effects of increased expression and GR promoter binding of the methyl-CpG-binding domain protein 2 (MBD2) and NGF1-A activation by maternal care have been implicated in this demethylation (Weaver et al., 2014).

Such processes of active demethylation maybe driven by the TET methylcytosine dioxygenase proteins TET1, TET2, and TET3, resulting in the oxidation of methylcytosine to hydroxymethylcytosine and further to formyl- and carboxylcytosine (Kohli and Zhang, 2013). Hydroxymethylcytosine is an epigenetic

mark that is most common in neuronal tissues and embryonic stem cells. It is considered an intermediate step in DNA demethylation. It is suggested that the formation of intermediate cytosine modifications leads to a less stringent recognition of the particular DNA sequence by the methylation maintenance proteins and methyl-binding proteins, possibly favoring a passive demethylation of these sites.

How the specificity of a potential TET-mediated demethylation is achieved remains unclear, but studies on interaction partners have shown that ten-eleven proteins can bind to other regulatory proteins, providing further evidence for sequence-specific regulation of DNA demethylation (Chen et al., 2013; Costa et al., 2013; Guilhamon et al., 2013).

Clearly, transcription factor binding to the DNA does not only facilitate the removal of methyl marks but also allows the directed de novo methylation. This is achieved by interaction with repressor proteins, chromatin remodeling enzymes, methyltransferases, and also small non-coding RNAs to open the chromatin and allow for remodeling, leading to either activation or repression of transcriptional activity (Marchal and Miotto, 2015; Meaney and Ferguson-Smith, 2010).

Signaling through Small Non-coding RNAs in Response to Stress

Another pathway that has been implicated in generating long-term epigenetic signatures of environmental exposure is the expression of small non-coding RNAs in the form of miRNAs and their subsequent targeting of stress-relevant pathways. A number of studies report stress-induced miRNA expression changes (Jung et al., 2015; Meerson et al., 2010; Rinaldi et al., 2010; Schouten et al., 2013; Smalheiser et al., 2011), and some studies are able to link these changes to pathways such as the HPA axis: Haramati et al. identified miR-34c to be upregulated in a stress-dependent manner. One of the targets of miR-34c is the 3′ UTR of the corticotrophin-releasing hormone receptor 1 (*crhr1*) gene leading to a decreased *crhr1* expression in response to stress-dependent miR-34c activation (Figure 3D) (Haramati et al., 2011). Micro RNAs also seem to be regulating the GR itself through post-transcriptional effects in rodents that are also sensitive to stress exposure (Jung et al., 2015). By activating specific miRNAs in the rodent brain, stress may thus influence the regulation of downstream genes that lead to an altered endocrine and behavioral response to stress. Human post-mortem brain studies implicate miRNA in depression with downregulation of specific miRNAs in the prefrontal cortex of these patients (Smalheiser et al., 2012). Interestingly, some of these changed miRNAs were predicted to target DNMT3B, which in turn has been shown to be upregulated in these samples. This suggests an interaction between the short-term regulation of gene transcription via small non-coding RNAs with the more long-lasting change conferred by DNA methylation. As outlined in the next section, confirmation of these models will require longitudinal studies that enable researchers to investigate the sequence of molecular changes in response to stress or trauma.

From a Short-Term Stress-Induced Imbalance to Long-Lasting Dysregulation and Disease

An altered mRNA transcription following exposure to environmental impact can be seen as a short-term compensatory reaction of the organism to maintain homeostasis and to overcome the environmental impact (McEwen and Gianaros, 2011). These immediate responses at the transcriptional level do not inevitably lead to long-lasting epigenetic changes. The long-term epigenetic changes in response to a qualifying environmental stressor require a sequence of short-term immediate molecular responses leading to long-lasting epigenetic adjustments. Moderators of these long-lasting epigenetic changes can be the quality, intensity, and timing of the stress exposure and interaction with genetic factors. An example for such concerted changes is the modification of the rodent arginine vasopressin (*avp*) promoter in response to maternal separation (Murgatroyd et al., 2009). Directly after a 10-day maternal separation period at postnatal day 10, the transcriptional activation of AVP is detectable with changes in phosphorylation of MeCP2 and protein occupancy but without changes in the DNA methylation. At this time point, the epigenetic memory has not been formed, and it is an intriguing question to ask if an early intervention e.g., by compensatory high maternal care, could prevent the transition from short-term MeCP2 phosphorylation to DNA methylation changes. The long-lasting epigenetic changes are established in a subsequent step, engraving the short-term transcriptional change by creating a long-lasting epigenetic memory by a reduced DNA methylation at the *avp* enhancer site for MeCP2 in the paraventricular nucleus of the hypothalamus (PVN) of early-life-stress-exposed mice. At this time point, the differential phosphorylation of the MeCP2 protein that served as an immediate molecular reaction to the environmental stimulus was not present anymore, and MeCP2 phosphorylation was indistinguishable from control mice. These data suggest that the immediate response via phosphorylation of MeCP2 is subsequently replaced by DNA methylation changes that persist over time. This example highlights that an understanding of factors leading to long-lasting modifications might help in improving our abilities to prevent and treat stress-related disorders. It also highlights the fact that longitudinal, prospective studies are imperative to delineate the temporal sequence of molecular events leading to disease, and that current studies, especially in humans, often do not provide more than snapshots of the respective disease conditions.

Differential Impact of Stress-Dependent Epigenetic Modifications Depending on Developmental Stage

Besides type and intensity of early life stress, the timing of the trauma is one of the most crucial factors determining epigenetic changes and psychopathological outcome. In general, epigenetic mechanisms are dependent on developmental stage and highly controlled as they play a major role in cell lineage determination but are also highly relevant in the adaptation of differentiated, post-mitotic neurons. Given the tremendous biological differences between developmental stages in human life with regard to epigenetic regulation but also hormonal regulation and neuronal connectivity, it is not plausible that there is a uniform epigenetic response to stress across the lifespan. The early life from pre-natal development until post-adolescence includes the development and subsequent maturation of neuronal circuits that support complex behavior, including language and cognition, but also pathways responsible for immune and hormone regulation that impact stress vulnerability or resilience, and this

period is more vulnerable to the detrimental effects of environmental stressors than any other periods in life (Fagiolini et al., 2009; Fox et al., 2010; Kanherkar et al., 2014; McEwen, 2008). Age dependence of stress vulnerability is illustrated by more recent studies on the well-established example of changed GR promoter methylation in response to maternal care or early life stress. These suggest that a shift in the timing of the stressor to adulthood does not lead to the same effects on the rodent I_7 GR promoter, or the human analog GR 1F promoter, methylation (Alt et al., 2010; Witzmann et al., 2012).

Developmental-stage-dependent vulnerability has also been documented in human studies, and we will now focus on human data in the following paragraphs. In extension of the rodent studies, comparable age-dependent findings are seen for stress-related changes on DNA methylation of *FKBP5*, where GR activation early in neuronal cell development, but not after differentiation, leads to lasting demethylation of GREs (Klengel et al., 2013). Another study from our lab investigated the molecular signature of childhood abuse in individuals exposed to adult trauma and suffering from PTSD (Mehta et al., 2013). We investigated peripheral gene expression and DNA methylation signatures in whole blood in these individuals and found evidence for distinct underlying biological mechanisms of peripheral blood gene expression changes between individuals suffering from PTSD exposed or not exposed to childhood trauma. We found not only differences in the pattern of gene expression between childhood-trauma-exposed versus non-exposed individuals but also a higher overall contribution of DNA methylation changes to the resulting expression patterns in early-life-traumatized individuals. The impact of early trauma on DNA methylation was concentrated in regions that are important for regulation of gene transcription, such as binding sites for enhancer or repressor proteins, but mostly outside of classical promoter regions and that included the 3′UTR and the gene body. Similarly, childhood, but not adult, socioeconomic status seems to impact epigenetic profiles (Lam et al., 2012).

These data suggest that the developmental stage of the exposure to environmental risk factors is an important determinant of their epigenetic effects. This dependence on the age variable is possibly related to the developmental trajectory of expression of epigenetic writers.

Direction, Genomic Localization, and Functional Effects of Trauma-Related Epigenetic Changes

Localization and Direction—Stress-Related Epigenetic Changes Affect the Whole Genome

A question that has not been addressed in this review up to now is whether there are unifying concepts of stress-related epigenetic changes, such as a common direction of effects throughout the genome (e.g., hyper- versus hypomethylation when focusing on DNA methylation), a clustering of these changes to specific genomic locations (e.g., promoter versus other regions), and, finally, whether these changes lead to concerted changes in gene expression in specific pathways.

The Direction of Stress-Induced Epigenetic Modifications

Most studies on stress-related epigenetic changes show a highly diverse response of the epigenome to stress rather than global up- or downregulation of DNA methylation or histone modifications. Follow-up studies in both rat and human post-mortem hippocampus tissue of GR promoter methylation with early environmental stressors initially investigated larger genomic regions surrounding the GR locus (McGowan et al., 2011; Suderman et al., 2012). These studies revealed that the early environmental exposure was associated with both hyper- and hypomethylation across larger stretches of DNA of about 100 kb, and this was seen both with maternal care in rats as well as with child abuse in humans. The overall distribution of hyper- and hypomethylation was also shown to be roughly equal. Investigating rat hippocampal tissue, Suderman et al. could show that DNA methylation changes were mirrored by changes in histone modification, with transcription-enhancing marks such as histone acetylation and methylation. DNA methylation increased in exonic regions, and DNA methylation decreased at promoter regions in rats exposed to higher maternal care. These studies have been taken to a genome-wide level by Labonté et al. (2012), who investigated the promoter methylation profiles in post-mortem hippocampal tissue of men having experienced childhood abuse or not and who compared these profiles with RNA expression profiles. Using cell-sorted neuronal and non-neuronal fractions for validation, the authors were able to show that differential methylation in promoter regions occurs mainly in the neuronal fraction, suggesting a significant impact of childhood abuse on neuronal epigenetic regulation, specifically in genes related to neuronal plasticity (Labonté et al., 2012). Moreover, Labonté reported twice as many hypermethylated promoters than hypomethylated regions. Other studies have investigated DNA from peripheral blood. Here, higher methylation levels were reported by Naumova et al. investigating methylation levels using the promoter-centric Infinium HumanMethylation27 BeadChip array in a small study of institutionalized children compared to controls (Naumova et al., 2012). These results are supported by results from our lab on patients with PTSD that report an increased number of hypermethylated regions with early trauma at gene loci, which show transcriptional differences with early trauma (Mehta et al., 2013). Another study, investigating the effects of early trauma in peripheral blood DNA using promoter-targeted methylated DNA immunoprecipitation (MeDIP), reported opposite effects, with more hypomethylated than hypermethylated regions (Suderman et al., 2014). These results have to be interpreted with care because all of the methods used are biased to certain genomic regions, especially CG dinucleotide (CpG)-rich promoter areas, and sample sizes were relatively small.

The genome-wide investigations of the epigenetic effects of early trauma seem to support concerted epigenetic changes with exposure to early life stress. Sudermann et al. report that with child abuse, hyper- versus hypomethylation clusters are of at least 1 Mb in peripheral blood (Suderman et al., 2014). In another genome-wide study on the effects of childhood maltreatment on peripheral blood using Illumina 450k methylation arrays, Yang et al. found that 74% of the differential methylated CpGs are located at low-methylation sites (i.e., sites with methylation levels below 20%). Those CpGs and medium-level methylated CpGs (between 20% and 80%) exhibit higher methylation levels in response to childhood maltreatment. In contrast highly methylated CpGs (above 80%) showed the

opposite effect, with lower methylation levels in maltreated children (Yang et al., 2013).

However, there is currently a lack of understanding how these diverse effects on epigenetic regulation may arise following an environmental impact. We can only speculate that inherent differences in the mechanisms driving the changes exist. As detailed above, different environments may lead to the activation of a specific or even multiple signal transduction cascades with epigenetic changes at regulatory regions in the genome. Subsequently, global changes in gene expression then may induce epigenetic changes across larger genomic regions.

The Genomic Location of Stress-Induced Epigenetic Changes

As with the direction of the effects, changes related to early stress are observed across all genomic regions and are not concentrated around CpG islands (regions > 200 bp with a high frequency of CpG sites, often located at promoter regions), for example. However, as noted above, all studies to date have to be interpreted with care because no comprehensive methylome-wide sequencing data have been generated from subjects with early environmental exposure, and all used techniques, even if assessing on a genome-wide level, are biased to specific areas in the genome by design. Nonetheless, these studies suggest that stress-related epigenetic changes may be preferentially located outside of classical CpG islands and promoter regions. We could show in peripheral blood DNA that childhood-abuse-related differentially methylated regions were enriched in regions outside the classical gene promoter, such as gene bodies, and were also less likely to be located in CpG islands, shores, and shelves (regions up to 2 kb and 4 kb, respectively, around CpG islands) and more likely in regions termed open sea (isolated regions in the genome that contain fewer CpGs) (Mehta et al., 2013). A similar distribution of environment-sensitive CpGs was found in a study investigating the effect of an in utero exposure to a natural disaster on the methylome of offspring, with < 10% of the detected changes in the CpG island and > 50% in open sea regions (Cao-Lei et al., 2014). The study by Yang et al. (2013) found low- to medium-methylated CpGs most sensitive to the impact of childhood maltreatment. These are located in regulatory regions such as downstream enhancers or CpG island shores, distinct and not classical promoters, or CpG islands that are often not methylated (Yang et al., 2013). In addition, Teh et al. (2014) showed that the effect of in utero exposure to variable environmental conditions results in differential methylation of variable methylated regions that tend to be located just outside of CpG islands, gene bodies, and intergenic regions (Teh et al., 2014). This suggests a role of more long-range regulatory elements in the epigenetic response to early environmental stressors such as child abuse, maternal care, and other early environmental factors. Here additional annotation with functional genomic elements, such as transcription factor binding sites, as well as better mapping of the 3D structure of chromatin will likely yield important mechanistic insights.

The Functional Effects on Gene Transcription

Epigenetic DNA regulation by methylation involves the following basic concept: increased methylation leads to a reduced mRNA expression, and decreased methylation leads to enhanced transcription. This only reliably occurs at sites close to the transcription start, surrounding the first exon. This is not the case in other genomic locations, with examples showing that an increased methylation can facilitate the expression of a certain gene depending of where methylation occurs (Wu et al., 2010), what type of methylation is installed (Sérandour et al., 2012), and which DNA-binding proteins are influenced by this mark (Niesen et al., 2005). This already implies that the effects of stress-related epigenetic changes, often located outside the promoter area, will present variable correlations of DNA methylation and gene expression changes. For example, our group could show that although DNA methylation at gene promoters negatively correlated with gene expression, the differentially methylated CpG sites in the gene body correlated with gene expression both positively as well as negatively, suggesting a bidirectional functional output of gene body methylation on gene enhancer and repressor regions. Similar patterns have been observed in other studies (Mehta et al., 2013; Teh et al., 2014).

An important point to note is that changes in DNA methylation due to early stress may not directly correlate with baseline gene expression but could lead to poised states that will determine future transcriptional responses following a specific environmental stimulus. Such a possibility has been suggested in a study by Lam et al. where variation in DNA methylation at specific CpG sites was not associated with baseline gene transcription but with the inflammatory response to ex vivo toll-like receptor stimulation in peripheral blood monocytes (Lam et al., 2012).

Another question is whether different types of DNA modifications may lead to different transcriptional effects, such as for methylcytosine versus hydroxymethylcytosine. Most studies currently do not distinguish between these two, and assays based on bisulfite conversion will measure both DNA methylation and hydroxymethylation. Here, future studies investigating both of these changes in parallel will be most informative. In addition, DNA methylation outside of CpG dinucleotides may add an additional layer of epigenetic code not investigated so far in the context of stress-related changes (Guo et al., 2014).

It also has to be acknowledged that epigenetic changes in stress-related psychiatric disorders clearly do not have the magnitude of DNA methylation changes in other fields, such as cancer research, and rather subtle changes in regulatory regions and specific neural cell types likely influence the behavioral phenotype. DNA methylation differences often below 10% are commonly seen, and these are close to the detection limits of the assays used. Moreover, the techniques available for detection of DNA methylation on a genome-wide level might introduce biases with regard to the magnitude of methylation changes and location by the inherent design of the assays. By using the 450k Illumina array or methods such as reduced representation bisulfite sequencing (RRBS) or tiling arrays, researchers might actually miss relevant signals that are located in areas that are not covered by the respective assay, as most of these assays are biased toward CpG-rich regions and promoter areas. In addition, most studies use mixed tissues with, most likely, only a fraction of cells actually being modified by stress and accounting for behavioral changes, thus diluting the effect of epigenetic modification. Here, a novel method that has successfully been

used to profile neurons is single-cell RNA sequencing, which could help in identifying the susceptible subset of cells (Zeisel et al., 2015).

Tissue Specificity of Stress-Induced Changes

Stress is an organism-wide response, with overlapping as well as distinct systems acting on different organs. Although, for example, effects of the stress hormone on the glucocorticoid receptor can affect a large number of different tissues, changes involving specific neural circuit activation will likely not have cross-tissue correlation. Here, we will discuss the question of whether peripheral tissue can be used to interrogate effects of stress in relationship to psychiatric disease.

Tissue Specificity Is Likely Dependent on Mechanisms by which Stress Induces Changes

Human neuropsychiatric studies bear the disadvantage that the primary tissue of interest, the brain, is usually not available. It is still controversial whether findings from peripheral tissues can be meaningful with regard to pathomechanisms. Studies now suggest that although a number of signaling pathways are very specific to the brain, others are common across tissues, and here extrapolations from one tissue to the other may be possible. One example is the activation of the GR, which is expressed in different isoforms across tissues and which is functionally active through transcriptional activation and repression of up to 10%–20% of all genes in the human genome (Oakley and Cidlowski, 2013). The activation of the HPA axis by stress leads to a global increase of cortisol. Although tissue-specific GR binding sites and poised states have been described, some sites show common activation across tissues (John et al., 2011). GR activation may thus lead to epigenetic adaptations across tissues. Ewald et al. (2014) could show that GR agonist exposure leads to correlated DNA methylation changes within the rodent *fkbp5* locus in both blood and brain and that DNA methylation levels in blood predicted DNA methylation and gene expression of *fkbp5* in the hippocampus. Interestingly, different intronic GREs were affected in blood and brain.

We have recently demonstrated that the allele-specific demethylation in *FKBP5* in peripheral blood cells following childhood abuse is paralleled by similar findings from in vitro studies of GR activation in a human hippocampal progenitor cell line. We observe the demethylation of a functional GRE in intron 7 of the gene, with the same three CpGs showing demethylation in peripheral blood cells with child abuse as well as with pharmacological GR activation in neuronal cells. Interestingly, this GRE, as identified by chromatin immunoprecipitation sequencing (ChIP-seq) (Wang et al., 2012), contains six CpG sites, of which three lie outside the three predicted consensus GRE binding sites and three lie either directly within or between consensus binding sites. Only the latter three show this demethylation, further suggesting the importance of GR activation in this process (Klengel et al., 2013).

Similar cross-tissue effects seem to be observed with the GR 1F promoter, and changes in DNA methylation within the NGF1-A binding sites are reported both in post-mortem hippocampus and peripheral blood (Turecki and Meaney, 2014; Zhang et al., 2013). Whereas the mechanism of this hypermethylation has been delineated in the hippocampus, it is not clear whether similar mechanisms, such as activation of NGF1-A and subsequent changes in DNA-binding proteins, also occur in blood cells. Genome-wide studies in rhesus macaques indicate that a number of tissues are also likely to be affected by differences in early-life-rearing experiences, with significant DNA methylation changes observed in both the prefrontal cortex and T cells. However, the number of specific sites actually overlapping between the two tissues was very limited (Provençal et al., 2012). Nevertheless, although these examples provide some evidence for a cross-tissue signature of, in this case, activation of GR and related stress pathways, specific signatures in peripheral tissues may not be observable for all psychiatric disorders.

In conclusion, although early experience most likely affects a number of tissues, and epigenetic effects could thus be observed not only in the brain, more studies are necessary to delineate which changes are tissue-specific and which are seen across several different tissues. Recent studies suggest that, in addition to peripheral blood cells, DNA derived from cheek swabs as well as saliva may also be used in epigenetic studies for psychiatric disease (Smith et al., 2015). Here, the collection is easy and often the only possible way to access DNA in children, but more studies about the potential usefulness of this tissue are warranted. In fact it has been shown that epigenetic profiles from both saliva and buccal epithelial cell DNA are distinct from those observed in peripheral blood in the same individual (Jiang et al., 2015; Smith et al., 2015). While the use of saliva or buccal cell DNA is promising and may allow large-scale epigenetic studies in epidemiological samples, it is important to acknowledge that in these tissues, there often is a mixture of epithelial and blood cells that needs to be accounted for. Bioinformatics methods have been developed with this aim in mind (Guintivano et al., 2013; Houseman et al., 2012; Jaffe and Irizarry, 2014; Lam et al., 2012).

Finally, peripheral blood cells may indeed give direct mechanistic insights into the brain as a number of studies have now implicated immune changes as one possible contributor to the pathophysiology of psychiatric disorders. For example, the most recent genome-wide association studies (GWAS) meta-analysis in schizophrenia showed an enrichment of variants in enhancer elements active in immune cells, supporting the hypothesis of immune-related pathologies as risk factors for the disorder (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). In fact, some studies suggest that immune changes can directly influence brain activity (Kronfol and Remick, 2000), and immune system activation can trigger stress-related disorders in a subset of patients (Felger and Lotrich, 2013). Stress-induced activation of the immune system leads to the release of cytokines and other signaling molecules that in turn can enter the brain and activate or modulate a broad range of neurotransmitter systems, neuroendocrine function, synaptic plasticity, and circuits that regulate mood and anxiety (Capuron and Miller, 2011). Such changes in immune cell composition but also activation status may directly reflect on the epigenetic profile. So, whereas correcting for changes in immune cell composition (Houseman et al., 2012) may be important for some questions, it could also hide effects where immune cell composition changes are causally related to disease.

Gene by Environment Interaction as a Unifying Concept

The past years have seen an increasing convergence of the exploration of genetic and environmental risk factors, acknowledging the well-known impact of the environment and, in particular, traumatic experiences in childhood on stress-related disorders (Heim et al., 2010) but also the inter-individual variability of outcomes following exposure to such events. This is highlighted in an ever-growing interest in gene by environment interaction (G×E) studies in psychiatry, a field that has gained momentum following a number of landmark studies by the team of Caspi and Moffitt (Caspi and Moffitt, 2006) and in which the interplay of usually common genetic variants with a broad spectrum of environmental factors on psychiatric outcomes was investigated. Although most of the current literature focuses on the combined effects of genetic variants and the detrimental effects of trauma, negative life events, and their long-term sequelae on mental health following the diathesis-stress model, G×E also includes by definition the genetic moderation of the effects of positive and protective environmental factors (Belsky and Pluess, 2013). A better understanding of the interplay of both harmful and advantageous as well as individual and shared environmental factors with individual genetic variation will contribute to explain individual differences of risk or resilience trajectories (Klengel and Binder, 2013; Manuck and McCaffery, 2014).

It has to be noted, though, that G×E studies that rely only on statistical interactions are fraught with possible methodological problems that need to be carefully considered when interpreting these studies (Almli et al., 2014; Dudbridge and Fletcher, 2014; Duncan and Keller, 2011; Keller, 2014; Manuck and McCaffery, 2014; Munafò et al., 2014). A molecular and systemic understanding of G×E will thus be important to develop improved methods and assessments to avoid many of the concerns that may lead to confounding in G×E studies. The integration of epigenetic mechanisms as outlined in the previous sections could support molecular models for G×E.

Given the replicated G×E of genetic variants in *FKBP5* with childhood abuse, we asked whether this interaction also influences the epigenetic response to childhood abuse. In fact, we were able to show that childhood abuse is associated with demethylation of *FKBP5* itself in a genotype-dependent response. Carriers of the allele that confers risk for later psychopathology also facilitate the demethylation of functional GREs in *FKBP5* when individuals were exposed to childhood trauma. In contrast, carriers of the protective genotype exhibit a more stable epigenetic configuration even when exposed to severe trauma. In this case, the SNP leads to differential 3D structure of the gene, with a GRE in intron 2 only coming into direct contact with the transcription start site in carriers of the risk allele. This is accompanied by higher *FKBP5* induction with stress and changes in the GR feedback that are associated with higher cortisol levels following stress. This increased activation of the GR following early trauma is then followed by a demethylation of a second GRE in intron 7 and a further increase in stimulated *FKBP5* transcription. In this case, the long-term epigenetic response is linked to the individual's genetic predisposition via subsequent systemic changes in the stress hormone system (Klengel et al., 2013). The importance of allele-specific epigenetic changes with environmental exposure is supported on a broader scale by the study of Teh et al. These authors investigated the genome and epigenome of neonates and found over 1,400 regions that were highly variable across individuals. A quarter of these were best explained by genetic variation only, while three-quarters were explained by an interaction between genotype and in utero environment, suggesting that allele-specific environmental effects occur throughout the genome and will only accumulate with age (Teh et al., 2014).

Severe stress and trauma may induce allele-specific epigenetic changes by different mechanisms. This can include very specific effects of sequence changes in a transcription factor binding site of a specific gene facilitating or impeding epigenetic effects following the activation of the transcription factors or direct changes of CpGs into other dinucleotides, with the possibility of propagating sequence-specific DNA methylation changes (Mill et al., 2008). Furthermore, DNA variants can alter the stress-induced activation of epigenetic writers. On the other hand, there may also be indirect effects, such as for FKBP5, where the DNA sequence changes lead to changes in the stress hormone system and to subsequent differences in the epigenetic effects of GR activation.

Epigenetic Modifications as a Potential Target of Psychiatric Therapy

The reversibility of environmentally induced epigenetic marks establishes the possibility to directly or indirectly interfere with these imprints to reverse or ameliorate disease status. It should be noted, however, that the use of drugs targeting epigenetic modifications in psychiatry is highly speculative at the moment. The bidirectional regulation of DNA methylation and other epigenetic marks leads to the question whether unidirectional drugs such as DNMT modulators or HDAC inhibitors can actually be used for the treatment of stress-elicited psychiatric disorders (Narayan and Dragunow, 2010; Szyf, 2009). At the same time, currently used antidepressants and related drugs, such as valproic acid, possess epigenetic effects (Göttlicher et al., 2001). Although the growing literature—in particular, from rodent models—suggests the involvement of epigenetic mechanisms in disease development, the location and direction of the epigenetic alterations are highly variable, challenging the idea of a single or even multiple epigenetic drugs that could restore the complex pattern of epigenetic states to a pre-disorder or resilient state. Among the major challenges is the fact that the specific targeting of epigenetic marks within the genome and even within the gene loci themselves is crucial to the functional outcome on gene regulation, as marks have opposing effects on transcription depending on their location. In addition, a safe manipulation of epigenetic states needs to be specific to the cell type and brain region that is responsible for disease development, which is often unknown. Although we learned much from using DNMT pan-inhibitors or HDAC inhibitors with respect to basic concepts of memory, learning, and environmental epigenetics (Bahari-Javan et al., 2014; Zovkic and Sweatt, 2013), these global modulators are not likely to deliver the temporal and spatial needs for a targeted epigenetic influence of psychiatric disorders. It is also possible that the administration of such drug inhibitors will be associated with severe side effects, possibly also

with long-term effects. It remains an open question whether different subtypes of HDACs and DNMTs will have distinct cell-specific roles in stress-related disorders, which may be addressed by subtype-specific inhibitors delivered to the cell type of interest. However, such specific drugs are lacking (Fischer, 2014).

Epigenetic modifiers might be used not only to change signatures in general or at specific genes but also to guide the epigenome toward a higher plasticity or to re-open windows of enhanced plasticity to facilitate the reversion of pathological epigenetic adaptations by conventional medications or psychotherapy (Sweatt, 2009). Evidence for a successful application of this strategy in humans was generated by using the HDAC inhibitor valproate for relearning absolute pitch, the ability to identify the correct pitch of a sound without reference. The authors propose that interfering with HDAC leads to a re-opening of a window of increased plasticity in the auditory system (Gervain et al., 2013). Finally, epigenetic studies may give insight into basic disease mechanisms and pathways disturbed in psychiatric disorders. These may then be directly targeted. If such an approach is tried with FKBP5, for which small molecule antagonists have been developed, that could reduce the genetically and epigenetically driven overactivity of this gene in trauma-exposed risk allele carriers. Early in vivo studies of these antagonists show promising behavioral effects in laboratory animals (Gaali et al., 2015).

In addition to being a potential therapeutic target, epigenetic marks could be useful in predicting and also monitoring therapeutic approaches (Guintivano et al., 2014a, 2014b; Powell et al., 2013; Roberts et al., 2014; Yehuda et al., 2013). For example, the peripheral blood methylation status at the GR locus that is altered in response to trauma exposure may predict the response to prolonged exposure psychotherapy, as proposed in a small pilot treatment study. This suggests that epigenetic changes in response to the traumatic event could predict further environmental modification, in this case by psychotherapeutic intervention (Yehuda et al., 2013). Roberts et al. observed an increasing DNA methylation at the serotonin transporter locus in individuals with anxiety disorders responding to cognitive behavioral therapy as compared to non-responders, who actually showed a decrease in DNA methylation (Roberts et al., 2014). Moreover, in a recent study by Powell et al., DNA methylation at the IL6 locus predicted response to classical antidepressant treatment in the Genome-Based Therapeutic Drugs for Depression (GENDEP) cohort, suggesting that epigenetic profiling before treatment could be used to reduce the likelihood of treatment failure by selecting the appropriate drug (Powell et al., 2013). Although these studies provide interesting evidence for potential biomarkers, careful interpretation of their relevance for central mechanisms is necessary.

Open Questions and Future Directions

Epigenetic modifications are emerging as an integral part of the molecular events leading to the development of stress-related psychiatric disorders, in particular in interaction with environmental adversities such as childhood trauma and genetic predisposition. We are only at the very beginning of understanding the temporal and spatial complexity of different layers of epigenetic regulation in psychiatric disorders, with animal models of stress-related disorders providing invaluable insights into the possible underlying mechanisms. Even though we understand basic mechanisms in reprogramming epigenetic patterns in response to environmental factors, the inherent complexity of these events in combination with genetic variations prevent, to date, a specific therapeutic intervention based on these principles. Most critical will be longitudinal studies delineating the chain of molecular events following stress and trauma leading to either risk or resilience and identifying the relevant cell types and tissues. Here, birth cohort studies with longitudinal samples of several tissues provide important insights but will need to be complemented by animal experiments for access to brain tissue.

Which Regions in the Genome Are Epigenetically Stress-Sensitive?

While we have focused on exemplary mechanisms and target genes, stress-induced activation of epigenetic mechanisms will remodel chromatin on a global scale but, nevertheless, in very specific stress-responsive regions. Here, the current progress in understanding the basic mechanisms of, e.g., DNA methylation need to guide efforts to apply these concepts to stress-related psychiatric disorders. For example, overall DNA methylation is reduced in regulatory regions compared to the majority of fully methylated cytosines across the genome, and Stadler et al. (2011) identified genomic regions that are characterized by a comparatively low density of CG dinucleotides (CpGs), open chromatin marks, and enhancer activity, which are prone to epigenetic modification by transcription factor binding. Here, the developmentally dependent activity of DNA-binding proteins may shape the DNA methylation profile in these regulatory regions of the genome (Blattler and Farnham, 2013; Feldmann et al., 2013; Stadler et al., 2011). However, the mechanisms underlying methylation patterns around specific transcription factor binding sites in regions with a low CpG density remain unclear (Baubec and Schübeler, 2014). Importantly, unbiased, genome-wide methods will be necessary to map these mechanisms, and integrative approaches combining the mapping of several epigenetic mechanisms with RNA expression in different cell types will be critical for a better understanding of these mechanisms (Kundaje et al., 2015). In the context of G×E, it will also be important to delineate how epigenetic mechanisms contribute to the regulation of long-range enhancers and the impact of genetic variants in these regions on altered chromosomal looping and transcription regulation. Long-range enhancers have gained increasing importance in schizophrenia (Bharadwaj et al., 2014; Roussos et al., 2014) and will likely be highly relevant in stress-related disorders.

Cell Specificity of Epigenetic Changes and Relationship to Systemic Changes

As mentioned above, all current epigenetic studies are using mixtures of cells. In the future, single-cell profiling, as possible for RNA expression (Zeisel et al., 2015), may lead to important insights, which changes are specific, and which are shared across different cell types and in extension tissues. In human studies, lack of online monitoring of changes in neurons will always hamper a deeper understanding of the dynamic epigenetic changes associated with the development of risk versus resilience to stress- or trauma-related disease. Here, patient-derived

pluripotent cell models and brain organoids, as briefly discussed below, may offer some insights. In addition, a number of studies have tried to correlate peripheral epigenetic changes with brain function using neuroimaging approaches. For example, increased promoter methylation of the serotonin transporter gene in peripheral blood predicted increased threat-related amygdala reactivity, and increased methylation of the same sites predicted decreased mRNA expression in post-mortem amygdala tissue (Nikolova et al., 2014). To date, however, the mechanistic insights from studies correlating peripheral epigenetic changes with brain imaging are limited. The development of positron emission tomography ligands based on compounds targeting epigenetic mechanisms, such as, for example, HDAC1 inhibitors, may also further our understanding of the contribution of these mechanisms in stress-related psychiatric disorders (Wang et al., 2013).

Manipulating Epigenetic Pattern through Genomic Engineering

The extension of using the protein-guided transcription activator-like effectors (TALEs) and the RNA-guided clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 systems to manipulate epigenetic marks may allow temporally and spatially controlled alterations of not only genetic sequences but also epigenetic signatures at DNA loci known to play a role in stress-related disorders (Maeder et al., 2013). By fusing epigenome-modifying proteins to the Cas9 protein, the RNA-guided system could allow the targeted manipulation of epigenetic states and thus the design of studies to understand the consequences of, for example, specific DNA methylation changes in response to early life stress. Given a successful control over off-target effects that would strongly limit the precise usage of this system, this may allow a multiplexed targeting of specific loci that are related to the pathophysiology of stress-evoked mental disorders, but it is unclear at the moment if the manipulation of even multiple loci across the genome is sufficient to revert the concerted and multilayered epigenetic changes across the genome in response to childhood abuse.

Induced Pluripotent Stem Cells—A Possible Model for Studying Neuronal Epigenetics in Stress-Related Disorders?

The inaccessibility of neuronal tissue is one major drawback in human epigenetic studies, although similarities between central nervous and peripheral tissue can be found, as discussed above, and immune cells may also be of primary interest. The generation of neuronal cells using induced pluripotent stem cells (iPSCs) also represents a promising future avenue for this kind of research. Brennand et al. were able to provide evidence that neurons created from fibroblast cells from schizophrenia patients recapitulate some cellular and molecular phenotypes related to schizophrenia (Brennand et al., 2011). While the genetic identity of the derived cells with the patient enables personalized investigations, it is unclear at the moment the degree to which the epigenetic profile will resemble that of the patient (Hjelm et al., 2013). Genetically driven epigenetic changes are likely to be recapitulated, possibly also as a response to different developmental programs. It is not known, however, whether acquired environmental changes will also be observed in the iPSCs (Roessler et al., 2014). In addition, especially for further differentiations to neurons, only a very short span of cell and organism development can be recapitulated in the dish, so epigenetic differences only triggered during later development or after birth may be very difficult to model in these cells. Overall, more research in this area will be necessary for a full assessment of the power of these model systems.

Epigenetic Effects In Utero and across Generations

Although this review focuses on postnatal epigenetic effects of stress, developments in recent years suggest that maternal stress during pregnancy can lead to early epigenetic programming of the fetus (Oberlander et al., 2008) and that parental stress exposure prior to conception may be transmitted to the next generation in the germline via epigenetic mechanisms (Dias and Ressler, 2014; Rodgers et al., 2013), with the findings of transgenerational inheritance in mammals being controversially discussed (Grossniklaus et al., 2013). So far, the relevance of ancestral environmental exposure for psychiatric disorders in decedents remains elusive. Such effects of in utero exposure to stress and transgenerational effects, while only convincingly documented in animals so far, need to be kept in mind when interpreting human studies where data are often collected cross-sectionally or do not include information on pregnancy or parental stress and trauma.

Overall, epigenetic mechanisms and their role in stress-related psychiatric disorders are a rapidly developing field, may yield important insights in the pathophysiology of these disorders, and may provide a mechanistic understanding of G×E. As further studies fill the gap in cell- and tissue-specific investigations over time and methylome-wide, the associated epigenetic changes may offer the possibility for development of biomarkers and novel treatment strategies for stress-related psychiatric disorders.

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