Epigenetic programming of the neuroendocrine stress response by adult life stress

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Abstract

The hypothalamic–pituitary–adrenal (HPA) axis is critically involved in the neuroendocrine regulation of stress adaptation, and the restoration of homeostasis following stress exposure. Dysregulation of this axis is associated with stress-related pathologies like major depressive disorder, post-traumatic stress disorder, panic disorder and chronic anxiety. It has long been understood that stress during early life can have a significant lasting influence on the development of the neuroendocrine system and its neural regulators, partially by modifying epigenetic regulation of gene expression, with implications for health and well-being in later life. Evidence is accumulating that epigenetic plasticity also extends to adulthood, proposing it as a mechanism by which psychological trauma later in life can long-lastingly affect HPA axis function, brain plasticity, neuronal function and behavioural adaptation to neuropsychological stress. Further corroborating this claim is the phenomenon that these epigenetic changes correlate with the behavioural consequences of trauma exposure. Thereby, epigenetic modifications provide a putative molecular mechanism by which the behavioural phenotype and transcriptional/translational potential of genes involved in HPA axis regulation can change drastically in response to environmental challenges, and appear an important target for treatment of stress-related disorders. However, improved insight is required to increase their therapeutic (drug) potential. Here, we provide an overview of the growing body of literature describing the epigenetic modulation of the (primarily neuroendocrine) stress response as a consequence of adult life stress and interpret the implications for, and the challenges involved in applying this knowledge to, the identification and treatment of stress-related psychiatric disorders.

Key Words

- epigenetics
- hypothalamic–pituitary–adrenal axis
- adult
- stress
- DNA methylation
- histone modifications
- microRNA

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Glossary

Restraint stress: a stress paradigm in which the animal is restrained in a confined space for a certain period of time, during which it is unable to move.

Social defeat: a stress paradigm that entails the (repeated) exposure of an animal to losing a confrontation with a dominant con-specific. It is most commonly established by the resident-intruder paradigm, in which the animal (the intruder) is repeatedly placed in the cage of a dominant animal (the resident) in a manner that allows for non-lethal contact.

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Chronic variable mild stress (CVMS): a paradigm in which the animal is exposed to various mild stressors for a prolonged period of time (usually twice daily for 14 consecutive days). Stressors include relatively mild sessions of social isolation, cold swim, cold isolation, wet bedding, food and water deprivation, overnight illumination, alteration of light-darkness cycle and restraint stress. All stressors are applied in a fixed order and only repeated twice to avoid habituation to the stressor.

Chronic variable stress (CVS): a paradigm in which the animal is exposed to various moderate stressors for a prolonged period of time (usually twice daily for 14 consecutive days). Stressors include social isolation, social crowding, warm swim, cold swim, cold isolation and cage rotation. All stressors are applied in a semi-randomized manner and only repeated twice, to avoid habituation to the stressor.

Chronic unpredictable stress (CUS): a paradigm in which the animal is exposed to various stressors for an extended period of time (usually once a day for 28 consecutive days). Stressors include cold swim, thermal environment, wet bedding, food and water deprivation, cage tilting, noise, overnight illumination and alteration of light-darkness cycle. All stressors are applied in a semi-randomized manner and only repeated twice, to avoid habituation to the stressor.

Introduction

Adequate responding to stress and restoration of homeostasis require a widespread activation of different response systems in the body. Crucial to the stress response is the neuroendocrine system, which tightly regulates adaptive processes following stress exposure (Miller & O'Callaghan 2002). The primary endocrine effectors of the neuroendocrine response are located in the paraventricular nucleus (PVN) of the hypothalamus, the anterior pituitary and the adrenal gland. This collection of structures, called the hypothalamic–pituitary–adrenal (HPA) axis, is critically involved in the regulation of a variety of body processes, including the immune system, energy storage and expenditure, digestion, mood and emotional responsivity to stress (Smith & Vale 2006). The neuroendocrine stress response should be adequate for coping with the specific stressor and should be of limited duration to prevent hyperactivity after stress cessation. Dysregulation of the HPA axis is associated with stress-related pathologies like major depressive disorder (MDD), post-traumatic stress disorder (PTSD), panic disorder and chronic anxiety (Tsigos & Chrousos 2002). Although depression pathology is linked to basal hyperactivation of the HPA axis (Parker *et al*. 2003, Swaab *et al*. 2005) and impaired negative feedback of the HPA axis (Burke *et al*. 2005), PTSD is thought to be characterized by increased sensitivity of glucocorticoid receptors (GRs), moderating enhanced negative feedback and overall decreased cortisol levels (Yehuda 2001). This endocrine dysregulation might be mediated by lasting neurobiological alterations caused by extreme or repeated stress exposure, especially in the case of PTSD, where trauma exposure is directly linked to the disease development.

Recent advances in stress research have implicated epigenetic modifications in the central nervous system as mechanisms by which environmental stimuli (such as stress) can induce long-lasting alterations in neurobiological systems (Provencal & Binder 2015*b*), including the neuroendocrine system (Auger & Auger 2013). The term 'epigenetics' refers to reversible chemical modifications to the chromatin structure that alter gene transcription without altering the DNA sequence. These include DNA methylation, DNA hydroxymethylation and histone modifications (i.e., methylation, acetylation and phosphorylation). Other important epigenetic modulators that influence protein expression are microRNAs (miRNAs), which act as translational repressors (Table 1). Although miRNAs do not alter chromatin structure and therefore technically do not follow the classical definition of epigenetics, they are, more often than not, considered important players in the epigenetic control of posttranscriptional gene expression. Altogether, these epigenetic modifications constitute important mechanisms by which transient environmental stimuli can induce persistent changes in gene expression and ultimately behaviour (Zovkic *et al*. 2013). However, the exact consequences of epigenetic modifications for gene transcription are not that straight forward, but seem to be context dependent and determined by both the location and the nature of the modification. For example, decreasing the accessibility of a gene regulatory element by DNA methylation could either decrease or increase nearby gene transcription, depending on whether a repressor or activator binds at that site (Zannas & West 2014).

It has long been understood that stress during early life can have a significant lasting influence on the development of neural and neuroendocrine systems, with implications for health and well-being in later life (Edwards *et al*. 2003, Chapman *et al*. 2004, Cougle *et al*. 2010, Tomalski & Johnson 2010). Alterations in epigenetic regulation have been suggested to contribute to this increased risk on neuropsychiatric disease by aberrant

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Table 1 Overview of epigenetic modifications.

Epigenetic modification	Definition
DNA methylation	DNA methylation (5-mC) is an epigenetic process in which a methyl group is added to nucleotides of DNA without any alterations to DNA sequence. In mammalian cells, this process predominantly occurs on the C5 position of cytosines in a cytosine-guanine dinucleotide (CpG) context. DNA methylation modulates gene expression by regulating accessibility of transcription factors to their binding sites and influencing chromatin structure. DNA methylation is mediated by a group of enzymes called DNA methyltransferases (Jones 2012)
DNA hydroxymethylation	The methylation state of DNA can be chemically modified by 10–11 translocation proteins, which oxidize the methyl group at the C5 position of cytosine and convert it to a hydroxymethyl group in a process called DNA hydroxymethylation (5-hmC) (Tahiliani *et al.* 2009). Hydroxymethylated DNA is a potential intermediary step in the demethylation pathway. Early findings suggest opposite roles of 5-mC and 5-hmC in nucleosome stability and regulation of gene expression (Mendonca *et al.* 2014)
Histone acetylation	Histone acetylation is the process whereby an acetyl functional group is transferred to the lysine residues in the N-terminal tail protruding from the histone core of the nucleosome. This modification transforms chromatin into a more relaxed structure that is associated with greater levels of gene transcription. Histone acetylation is mediated by histone acetyltransferases and histone deacetylases (Gräff & Tsai 2013)
Histone phosphorylation	Histone phosphorylation involves the addition of a phosphate functional group to serine, threonine, and tyrosine residues in the histone N-terminal tail. The best-known function of this process occurs during DNA breakage, when phosphorylated histone H2A(X) demarcates large chromatin domains around the damaged area. However, recent findings have also linked this epigenetic mark to transcriptional activation of a variety of genes, most often related to cell growth and proliferation (Rossetto *et al.* 2012)
MicroRNAs	MicroRNAs (miRNAs) comprise species of short non-coding RNA that can negatively control target gene expression posttranscriptionally. As such, miRNAs can not only influence the translation of a plethora of different genes directly, but they can also target the expression of genes that control epigenetic pathways, like DNMTs and HDACs (Sato *et al.* 2011)

DNMT, DNA methyltransferase; HDAC, histone deacetylase.

gene expression and cell differentiation during early developmental stages (Crews & Gore 2011, Maccari *et al.* 2014). Early in development, each cell in the body starts placing epigenetic marks during differentiation under the influence of perinatal environmental cues, with the goal of establishing an adaptive long-term phenotype that meets the probable demands later in life (Migicovsky & Kovalchuk 2011). This process, i.e., transdifferentiation (Waddington 1957) or epigenetic reprogramming (Ho & Tang 2007), may last for weeks, months and even years, depending on the cell or tissue type. Altered environmental cues (e.g., stress) may therefore greatly affect brain development, as well as regional gene expression throughout life, in an attempt to meet environmental demands. Depending on the environment of later life, these epigenetic changes can prove to be either adaptive or maladaptive, thereby protecting from or increasing the risk of mental disease (McClelland *et al.* 2011, Provencal & Binder 2015*a*). Although it is thus clear that there is a window of sensitivity for environmentally induced epigenetic changes during perinatal development, influencing risk on psychopathology, evidence is accumulating that epigenetic plasticity also extends into adulthood (Miller & Sweatt 2007, Lubin *et al.* 2008, Feng *et al.* 2010, Miller *et al.* 2010). It has been shown that psychological trauma during adulthood can induce epigenetic changes that affect brain plasticity, neuronal function and behavioural adaptation to neuropsychological stress (Hunter *et al.* 2009, Roth *et al.* 2011). Hence, these epigenetic changes may provide a molecular mechanism for the phenotypical development observed e.g., after trauma exposure in PTSD, explaining how phenotype and transcriptional potential can change drastically and long lastingly in response to environmental challenges, even when experienced in adulthood. As such, more recent advancements in the fields of epigenetics have focused on the presence of stress-mediated epigenetic modifications in adulthood. The ability of stressful events to affect epigenetic regulation in the brain has been illustrated in fear conditioning and extinction paradigms in rodents, where contextual fear learning induced altered methylation patterns in memory- and plasticity-related genes (Miller & Sweatt 2007, Lubin *et al.* 2008). Altered hippocampal DNA methylation levels have also been observed in rodent models for PTSD (Chertkow-Deutsher *et al.* 2010, Roth *et al.* 2011). These modifications of DNA transcription were shown to be persistent (Malan-Muller *et al.* 2014) and even transmissible across generations (Yehuda *et al.* 2014, Dias *et al.* 2015), underlining their importance as mediators of the imprinting of stressor experience on

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brain and behaviour. Enhancing our understanding of the epigenetic mechanisms that occur following stress exposure has far-reaching clinical potential. Stress exposure in adulthood not only contributes to the development of stress-related mental disorders, it can also precipitate or perpetuate other psychiatric disorders (e.g. addiction, dementia and schizophrenia) and can negatively affect the course of non-psychiatric conditions like cancer and cardiovascular disease (Zannas & West 2014). As such, being able to improve the ability to treat neuropsychiatric disorders, would not only decrease world-wide stress-related disability, but would also significantly reduce the ever-increasing health care costs.

Here, we provide a review of recent studies in humans and rodents on epigenetic modulation of the (primarily neuroendocrine) stress response as a consequence of adult life stress. We first summarize evidence for the global changes in epigenetic markers as a consequence of stress exposure in adulthood in rodents and humans. Although (chronic) stress exposure has been clearly linked to increased risk on MDD (Lueboonthavatchai 2009), studies in depressed patients were left out of consideration here, as prior stress exposure is no prerequisite for MDD diagnosis and resulting pathology can therefore not be causally linked to the experience of (adult) life stress (as is the case for PTSD). We then offer an overview of scientific evidence for stress-induced epigenetic alterations in HPA axis function and in stress-related neurotransmitter systems. Finally, we discuss the implications of these data for and the challenges of applying this knowledge to the identification and treatment of stress-related psychiatric disorders.

Stress-related general epigenetic changes

Human studies

Blood samples of PTSD patients constitute the primary evidence for long-lasting epigenetic modifications due to (adult) stress exposure in humans. Studies have indicated that PTSD patients display increased levels of trimethylation in histone 3 lysine 4 (H3K4), H3K9 and H3K36 in peripheral blood mononuclear cells (Bam *et al*. 2016), suggesting altered activity of histone methyltransferases (HMTs) and demethylases (HDMTs), which most likely affects the expression of a plethora of genes. Moreover, a global increase in DNA methylation at thousands of DNA CpG sites was found to be associated with PTSD (Smith *et al*. 2011). These changes were independent of age, ethnicity and, most importantly, early life stress, suggesting that stress during adulthood can alter global DNA methylation patterns, likely through differential regulation of DNA methyl transferases (DNMTs).

Although the aforementioned studies relied on retrospective data and thus were unable to demonstrate a causal relationship between stress exposure and the observed epigenetic profiles, a recent longitudinal study by Sipahi and coworkers (Sipahi *et al*. 2014) actually did indicate such a causal link. Here, pre- and post-trauma DNA methylation profiles were compared in PTSD patients and age-, gender- and trauma exposure-matched controls. Trauma exposure was found to be associated with increased DNA methylation at multiple CpG loci in *DNMT1*, *DNMT3A* and *DNMT3B* genes. However, remarkably, these epigenetic responses to trauma did not differ between healthy subjects and patients, except for the increased *DNMT1* methylation, which was only observed in patients, suggesting that the majority of these epigenetic changes occurred in response to stress regardless of eventual behavioural symptoms. Moreover, pre-trauma DNA methylation was higher in the patients compared to controls at a single *DNMT3B* CpG site, reflecting a pre-existing risk factor for the development of PTSD in response to trauma. This finding highlights the importance of longitudinal studies for the identification of (epigenetic) risk markers for PTSD and to distinguish these from pathology-related epigenetic changes that should be targeted in evidence-based interventions (Box 1).

Besides these well-known alterations in gene methylation patterns, recent studies of the epigenetic regulation of the stress response have increasingly implicated miRNAs as important mediators of environmentally induced alterations in gene expression. miRNA expression levels in rodents and human cells have been found to be altered in response to various environmental factors, such as light, sound, nutrients, drugs and stress (Codocedo & Inestrosa 2016). Preliminary results have demonstrated the upregulation of several serum miRNAs directly after an acute social stress task in healthy participants (Vaisvaser *et al*. 2016) and have associated transiently altered expression of serum miRNAs with chronic academic stress (Honda *et al*. 2013). Abnormalities in miRNA expression have also been implicated in PTSD, with several miRNAs being significantly downregulated in PTSD cases vs age-matched healthy controls (Zhou *et al*. 2014). Lower expression of DICER1, an enzyme that contributes to the generation of mature miRNAs, has been proposed as a molecular mechanism for this decrease in global miRNA levels (Wingo *et al*. 2015). Expression of DICER1 and other DICER-like proteins themselves might

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Box 1 Epigenetic contributions to individual stress vulnerability.

Stressful life events (SLEs), caused by environmental, psychological, or social situations, are important risk factors for the development of neuropsychiatric disorders, including MDD, PTSD, and anxiety disorders (Breslau 2002). While an estimated 90% of individuals in the general population are faced with one or multiple SLEs at some point in their lives, only a small percentage of these individuals ultimately develop psychiatric symptoms. This implicates inter-individual differences in the underlying mechanisms constituting (natural) vulnerability or resilience to stress-induced pathology (Kessler *et al*. 2005). Influential studies on monozygotic twins have demonstrated that stress vulnerability can be explained partially (30–70%) by genetic variation, mainly mediated by single nucleotide polymorphisms (Afifi *et al*. 2010, Pitman *et al*. 2012). In addition, epigenetic patterns, either inherited or resulting from the cumulative environmentally-induced alterations that occurred throughout life, can shape vulnerability (i.e., the induction of pathological processes following stressor exposure) and resilience (i.e., the absence of psychiatric symptoms despite stressor exposure) to the development of psychopathology following future stressors (Zannas & West 2014). As such, neuropsychiatric disorders which develop during adulthood are most likely caused by a combination of pre-existing genetic and epigenetic vulnerability factors and alterations that are caused as a consequence of adult life stress exposure itself (Jirtle & Skinner 2007), as suggested by the diathesis-stress model for psychiatric illnesses (Monroe & Simons 1991) and the three-hit concept of vulnerability to stress-related mental disorders (Daskalakis *et al*. 2013). In line with this idea of differential (pre-existing) epigenetic patterns reflecting vulnerability, DNA methylation of *SKA2* and *BDNF* prior to trauma exposure was found to predict suicidal behaviour and PTSD symptomatology (Kang *et al*. 2013, Kaminsky *et al*. 2015, Clive *et al*. 2016), while methylation of *SLC6A4* (Swartz *et al*. 2016) and *GRIN1* (Weder *et al*. 2014), which encodes subunit zeta-1 of the N-methyl-D-aspartate (NMDA) glutamate receptor, predicted depression. Furthermore, other human studies have linked the basal state of the DNA methylome to substance abuse (Andersen *et al*. 2015), aggression (Schechter *et al*. 2017), and depressive behaviour (Zhao *et al*. 2013).

also be epigenetically regulated, as is suggested by multiple studies investigating RNA-directed DNA methylation in plants (Boyko *et al*. 2010, Pumplin *et al*. 2016).

Although examination of DNA extracted from peripheral blood from patients has provided us with important indications of epigenetic changes induced by stressful life events, stress-related disorders are disorders of the brain. Inter-individual variation in whole blood is not a strong predictor of inter-individual variation in the brain (Hannon *et al*. 2015), and epigenetic patterns vary substantially across functionally distinct brain regions (Davies *et al*. 2012), making that blood- or saliva-based epigenetic studies provide only limited information on the actual pathological neural processes. Therefore, additional research in brain tissue is important for assessing the epigenetic plasticity of neural cells as a consequence of adult life stress. Human post-mortem studies are most suitable in this respect, but data available is limited as these studies face multiple practical issues in the collection of tissue from a sufficient amount to appropriate subjects, together with a detailed subject history of SLEs, stress-related pathology, and use of medication. Longitudinal studies (Yehuda 2004), as well as increased storage and application of human post-mortem data in biobank tissue repositories, like the USA's National Institutes of Health Neurobiobank (Nichols *et al*. 2014), are necessary to increase insight in the brain region-specific epigenetic profiles associated with stress-related psychopathology.

Animal studies

A useful remedy to study the epigenetic effects of stress exposure associated with the pathology of stress-related mental disorders in brain tissue is the use of animal models. Animal models provide us with a means to study stress in organisms that (i) have a homogeneous genetic and environmental background, (ii) can be exposed to standardized stress paradigms in a controlled fashion, (iii) can easily be longitudinally studied and (iv) allow for more invasive (direct) measurements of brain tissue rather than peripheral blood. Therefore, animal studies allow for the investigation of the causal relationship between stress exposure and changes in the epigenome and thereby to dissect whether epigenetic patterns reflect psychological states (as a consequence of stress) that contribute to psychopathology (Box 2). When studying the stress response in rodents, multiple brain regions are of importance. First of all, the regions involved in the HPA axis are relevant. These include the paraventricular nucleus (PVN) of the hypothalamus, which contains neuroendocrine neurons that synthesize and secrete corticotropin-releasing hormone (CRH) and vasopressin, and the pituitary,

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Box 2 Epigenetic contributions to a stress-related phenotype.

When investigating the epigenetic 'backbone' of stress-related disorders to improve treatment, it is important to consider the causal relationship between the epigenetic signature and the observed behavioural phenotype. Yet, it is difficult to establish (i) which epigenetic marks are directly linked to a certain stressful event (or instead reflect inborn differences (Box 1)) and (ii) which epigenetic marks directly contribute to pathology, by mere *post-hoc* comparisons in human studies. However, rodent studies can be specifically designed to yield information about the exact factors contributing to a stress-related phenotype. Two important contrasts are studied (Fig. 1):

(i) Stressed vs control. Half of the animals from a genetically homogeneous group undergo a certain stress procedure, while the other animals receive a sham procedure. Afterwards, differences in epigenetic regulation between the two groups are assessed. Notably, the observed differences reflect epigenetic changes that can be directly linked to stress exposure, and are likely reflective of the mean behavioural differences between the stressed and control animals, but not necessarily directly related to any stress-induced phenotype.

(ii) Resilient vs vulnerable. All animals from a group undergo the same stress procedure and are tested on stress-related symptomatology afterwards. The behaviourally (most) resilient animals are compared to the behaviourally (most) vulnerable animals to distinguish potential adaptive from maladaptive epigenetic changes as a consequence of stress exposure. More so than in the stressed vs control contrast, this contrast links epigenetic signature directly to the behavioural phenotype (i.e., psychopathology). However, the observed epigenetic signature is not necessarily linked to any alterations induced by the stress procedure in itself, as the animals' epigenetic profiles might have already been distinct before the procedure (and reflect innate susceptibility (Box 1)). Still, studying which epigenetic marks underlie the behaviourally adaptive responses of the resilient animals that distinguish them from the behaviourally maladaptive ones, may provide useful starting points for treating stress-related disorders.

which secretes adrenocorticotropic hormone (ACTH) (Smith & Vale 2006). Limbic structures of the forebrain are involved in the regulation of the HPA axis, with the hippocampus and prefrontal cortex (PFC) contributing to glucocorticoid-induced feedback inhibition of the HPA axis, while amygdalar binding of glucocorticoids has been associated with its feed-forward excitation (Herman *et al.* 2005). As the neuronal populations in these regions also form the respective anatomical substrates for emotional responding, memory formation, and emotion regulation, they may serve as a link between the stress system and the emotional and cognitive abnormalities observed in neuropsychiatric disorders (McEwen 2000). Besides these 'classical' regulators, an emerging neurobiological substrate of the stress response is the nucleus accumbens (NAc), where CRH facilitates cue-elicited motivation and social bonding through dopaminergic transmission (Pecina *et al.* 2006). Chronic stress has been reported to induce drastic neurochemical alterations in the NAc, leading to a depressive phenotype (Di Chiara *et al.* 1999).

General epigenetic effects of acute stress exposure Research in rodents has indicated that epigenetic modulation and corresponding changes in gene expression as a consequence of stress exposure critically depend on the frequency of the stressor (Harbuz & Lightman 1992). For example, differential histone methylation patterns in rat hippocampus were observed resulting from either 1 day (acute), 7 days (subchronic), or 21 days (chronic) of restraint stress (Hunter *et al.* 2009). H3K9 and H3K27 trimethylation, associated with transcriptional silencing (Barski *et al.* 2007, Karmodiya *et al.* 2012), were increased by both acute and subchronic stress, but decreased by chronic stress. Conversely, H3K4 trimethylation, a known activator of gene transcription (Barski *et al.* 2007), was unaffected by acute and subchronic stress, but significantly increased after chronic exposure. It could be hypothesized that general transcriptional silencing in response to acute stress exposure may avoid the brain from overreacting to the stimulus, whereas activating specific genes in response to chronic stress may allow the brain to properly adapt to the new stressful environment. However, no behavioural data were collected in this study, leaving the functional (i.e., behavioural) relevance of these alterations open for future investigation. Interestingly, repetition of the acute stressor seems to increase its potential to evoke epigenetic alterations. Four consecutive 15-min sessions of social defeat stress on one day, but not one single 15-min session, increased hippocampal H3 acetylation in a rat model of social defeat, accompanied by increased depressive-like behaviour (Hollis *et al.* 2010). However, H3 acetylation in the defeated animals returned to baseline levels 72 h after the stress episode, even though the depressive behaviour remained present for at least 6 weeks. While this might argue

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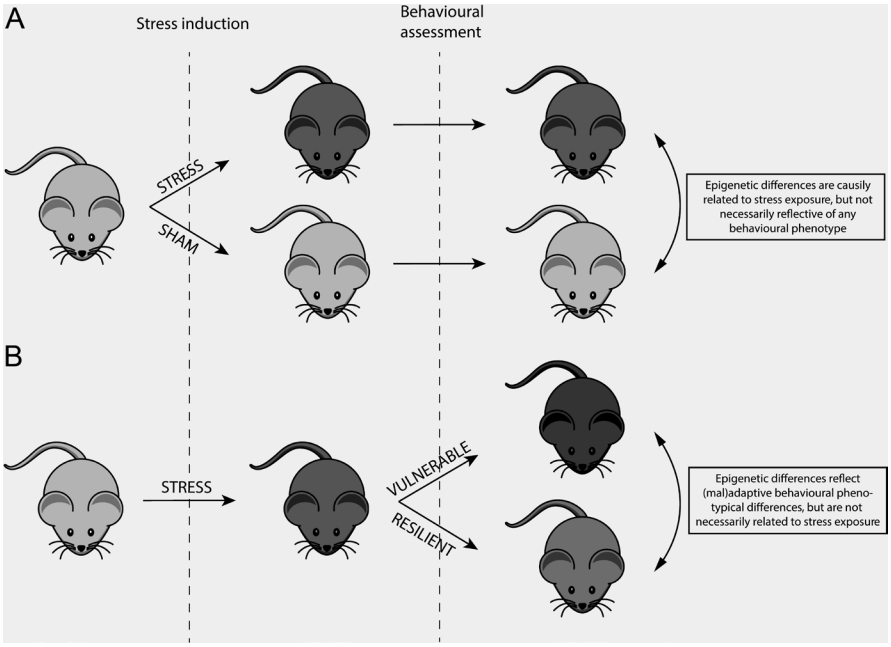

Figure 1
Animal models of stress. (A) Stressed vs control contrast: half of the animals from a genetically homogeneous group undergoes a certain stress procedure, while the other half receives a sham procedure. (B) Vulnerable vs resilient contrast: all animals from a group undergo the same stress procedure and are tested on stress-related symptomatology afterwards. The behaviourally most resilient animals are compared to the behaviourally most vulnerable animals.

against the histone modification as a potential underlying mechanism for the behavioural profile, transient changes in histone acetylation have previously been proposed to induce long-term changes in gene activity (Tsankova *et al.* 2006, Shahbazian & Grunstein 2007) and behaviour (Weaver *et al.* 2004) by inducing transcription of genes that influence the transcription of other downstream targets that are more long-lasting. This emphasizes that their modulation, albeit transiently, can have long-lasting consequences. In line with this modulatory role for stressor frequency, Renthal *et al.* (2007) showed that a single 10 min session of social defeat stress was insufficient to alter levels of the histone deacetylases (HDACs) 1, 2, 3, 4, 5 and 9 in the NAc of adult mice, but that a 10-day repetition of the paradigm downregulated HDAC5 in the NAc by almost 25%. This regulation of HDAC5 expression likely contributed to the behavioural consequences of the stressor, as in this same study it was found that HDAC5 knockout mice developed more severe social avoidance and anhedonia in response to the stress paradigm than wild-type littermate controls. Interestingly, knockout and wild-type mice did not differ in their behavioural responses to an acute defeat episode, indicating that HDAC5 is involved in the epigenetic regulation of behavioural adaptations to chronic, but not acute, stress. These findings suggest that the regulatory systems involved in the brain's innate response to stress differ between acute and chronic exposure. This is especially interesting for understanding vulnerability to PTSD, as both acute (e.g., violent personal assault and severe car accidents) and chronic stress (e.g., war and child neglect) exposure can precipitate PTSD-associated psychopathology (Javidi & Yadollahie 2012).

The effectiveness of acute stress to induce epigenetic changes seems to not only depend on stressor frequency, but also on stressor dimension and severity, as 15 min of forced swimming and 30 min of predator exposure, but not 3 min of ether vapour exposure or 4 h of cold exposure, were found to increase H3 phosphorylation in the rat dentate gyrus (DG) (Bilang-Bleuel *et al.* 2005). One hour of acute restraint stress also appeared to be sufficient to significantly decrease global DNA methylation levels in rat hippocampus, medial prefrontal cortex (mPFC), and periaqueductal grey (Rodrigues *et al.* 2015). Possibly, stressors with a strong psychological component (such as restraint and predator exposure) might be more effective at inducing epigenetic changes than primarily physical stressors (such as cold and vapour exposure) (Bilang-Bleuel *et al.* 2005).

Epigenetic involvement in the persistent behavioural consequences induced by acute stressors is also apparent

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in the formation of long-lasting, recurring traumatic memories, characteristic for PTSD (Parsons & Ressler 2013). Animal models have identified a critical contribution of epigenetic modifications in the hippocampus and amygdala to the encoding and expression of fear memory (Gudsnuk & Champagne 2012, Stankiewicz *et al*. 2013). DNMT inhibition in the rat hippocampal CA1 region (Miller *et al*. 2008) and lateral amygdala (Monsey *et al*. 2011) following fear conditioning was shown to disrupt the consolidation of contextual and cued fear, respectively. This indicates an important role of DNA methylation in trauma memory formation. Moreover, histone acetylation, especially in hippocampal H3 (Levenson *et al*. 2004) and H4 (Peleg *et al*. 2010), as well as amygdalar histone trimethylation of H3K4 (Gupta *et al*. 2010), have been found to also promote fear encoding. Extensive reviews describing the involvement of epigenetic mechanisms in fear memory formation have been performed by Roth *et al*. (2010), Zovkic *et al*. (2013), Kwapis and Wood (2014), Rudenko and Tsai (2014), and Blouin *et al*. (2016).

All in all, acute stress is able to induce changes in histone methylation and acetylation, and DNA methylation in the brain, but seemingly most pronouncedly when the stressor is frequent and severe enough. This seems logical, as epigenetic modulation serves to optimally prepare and adapt the organism for future reoccurrences of the same stressor and to assist coping with similar stressful conditions. The occurrence of epigenetic alterations in response to a specific, acute stressor can therefore be expected to increase when the likelihood that the stressor will occur more often increases, or when the stressor is so severe that it is of utmost importance to sufficiently prepare for it. One could even speculate that it is safer to avoid epigenetic modulation after an acute stressor to prevent the induction of a potentially maladaptive long-term phenotype, until it becomes apparent that the organism needs to durably adapt to changes in the environment.

General epigenetic effects of chronic stress exposure The epigenetic effects of chronic stress have been more elaborately studied. At the histone level, many changes in methylation and acetylation status have been found following repeated stress exposure. Wilkinson *et al*. (2009) observed increased accumbal H3K9 and H3K27 dimethylation in rats exposed to either 10 days of social isolation or social defeat stress compared to controls, which was associated with depressive-like avoidance behaviour. As animals that were behaviourally resilient to the social avoidant phenotype displayed histone methylation levels resembling those of control animals, these epigenetic effects seem to be directly related to the behavioural consequences of this chronic stressor. Both the increase in histone dimethylation and the avoidant phenotype remained stable 28 days post-stress-termination, indicating that the changes are rather long-lasting. Moreover, the increases in methylation level were significant even after averaging across the entire genome, lending credence to the idea that widespread stress-induced epigenetic changes in the NAc occur throughout the entire genome. In contrast to the increased histone methylation in the NAc, 10-day socially defeated animals were shown to display decreased global DNA methylation levels in the mPFC (Elliott *et al*. 2016), which were accompanied by an anxious phenotype. This reduction in global methylation levels was associated with a decreased expression of mPFC DNMT3A. Further confirming the region-specific nature of the epigenetic changes in the brain, DNMT3A was upregulated in the central nucleus of the amygdala (CeA), while DNMT3B levels, which were not altered in the mPFC, were downregulated in this region. Other studies have reported that DNMT3A is upregulated in the NAc (LaPlant *et al*. 2010) and downregulated in the hippocampus (Hammels *et al*. 2015) of defeated vs control mice. Additionally, DNMT3B was found to be reduced in the paraventricular nucleus (PVN) of the hypothalamus (Elliott *et al*. 2010) of vulnerable vs resilient mice after chronic social defeat. Chronically stressed animals also show differential histone acetylation patterns when compared to controls. Ferland and Schrader (2011) reported on decreased rat hippocampal H3K9 and H4K12 acetylation as a consequence of 14-day chronic variable stress (CVS). Application of HDAC inhibitors to hippocampal slices induced a stronger increase in histone acetylation in the CVS animals compared to the controls, implying higher HDAC activity as a consequence of chronic stress. Similar decreases in hippocampal H3K9 and H4K12 acetylation were observed in rats following 28 days of chronic unpredictable stress (CUS) (Liu *et al*. 2014), which was accompanied with a significant increase in HDAC5 in hippocampal tissue. Interestingly, HDAC5 was found to be downregulated in the amygdala (Sterrenburg *et al*. 2011), as well as the NAc (Renthal *et al*. 2007) in chronically stressed rats, again pointing towards region-specific epigenetic modulations. Lastly, HDAC2 was found to be downregulated by 10-day social defeat stress in the NAc of defeated vs control mice, coinciding with increased accumbal H3K14 acetylation in the NAc (Covington *et al*. 2009) and in the PVN of social avoidant vulnerable compared to resilient mice (Elliott *et al*. 2010)

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(Table 2 for an overview of all reported region-specific stress-induced epigenetic changes).

Rodent models have also demonstrated altered region-specific miRNA levels in response to both acute (Rinaldi *et al*. 2010, Haramati *et al*. 2011, Mannironi *et al*. 2013, Hosoya *et al*. 2016) and chronic stressors (Meerson *et al*. 2010, Babenko *et al*. 2012, Volk *et al*. 2016). Moreover, altered miRNA expression levels have been observed as a consequence of a model for PTSD-induction in rats (Balakathiresan *et al*. 2014) and have been proposed as mediators of resilience to chronic stress (Issler *et al*. 2014, Higuchi *et al*. 2016). The regulation of non-coding RNAs, including miRNAs, in animal models of PTSD has been extensively reviewed by Schmidt *et al*. (2015).

Table 2 Overview of stress-induced general epigenetic changes in the rodent brain.

Species	Paradigm	Measurement post-stress	Brain region	Epigenetic changes		References
Acute stress						
Rat	Restraint stress (30 min)	Immediately	Hip (DG, CA1)	H3K9me3 ↑ H3K27me3 ↓		Hunter *et al*. (2009)
Rat	Restraint stress (1 h)	1 day	Hip, PAG	5-mC ↓		Rodrigues *et al*. (2015)
Rat	Restraint stress (2 h)	Immediately	Hip	H3ac ↓		Fuchikami *et al*. (2009)
Rat	Social defeat (4 x 15 min)	Immediately	Hip	H3ac ↑		Hollis *et al*. (2010)
Rat	Forced swim (30 min) Predator stress (15 min)	1 day	Hip (DG)	H3ph ↑		Bilang-Bleuel *et al*. (2005)
Chronic stress						
Rat	Restraint stress (21 days)	1 day	Hip (DG)	H3K9me3 ↓ H3K4me3 ↑		Hunter *et al*. (2009)
Mouse	Social isolation (10 days) Social defeat (10 days)	28 days	NAc	H3K9me2 ↑ H3K27me2 ↑		Wilkinson *et al*. (2009)
Mouse	Social defeat (10 days)	28 days	Hip	H3K27me2 ↑		Tsankova *et al*. (2006)
Mouse	Social defeat (10 days)	1 day	mPFC	5-mC ↓	DNMT3A ↓	Elliott *et al*. (2016)
			Amygdala (CeA)		DNMT3A ↑ DNMT3B ↓	
Mouse	Social defeat (10 days)	Immediately	Hypothalamus (PVN)		DNMT3B ↓	Elliott *et al*. (2010)
Mouse	Social defeat (10 days)	10 days	NAc		DNMT3A ↑	LaPlant *et al*. (2010)
Mouse	Social defeat (10 days)	1 day	Hip (DG)		DNMT3A ↓	Hammels *et al*. (2015)
Mouse	Social defeat (10 days)	1 day	NAc		HDAC5 ↓	Renthal *et al*. (2007)
Mouse	Social defeat (10 days)	10 days	NAc	H3K14ac↑	HDAC2 ↓	Covington *et al*. (2009)
Mouse	Social defeat (10 days)	Immediately	PVN		HDAC2 ↓	Elliott *et al*. (2010)
Rat	CUS (21 days)	1 day	Hypothalamus	H3K9me3 ↓		Wan *et al*. (2014)
Rat	CUS (28 days)	1 day	Hip	H3K9ac ↓ H4K12ac ↓	HDAC5 ↑	Liu *et al*. (2014)
Rat	CVMS (14 days)	1 day	Amygdala (CeA)		HDAC5 ↓	Sterrenburg *et al*. (2011)
Rat	CVS (14 days)	1 day	Hip (DG, CA3)	H3K9ac ↓ H4K12ac ↓		Ferland and Schrader (2011)

5-mC, methylated DNA; ac, acetyl; CeA, central nucleus of the amygdala; CUS, chronic unpredictable stress; CVMS, chronic variable mild stress; CVS, chronic variable stress; DG, dentate gyrus; DNMT, DNA methyltransferase; H, histone; HDAC, histone deacetylase; Hip, hippocampus; K, lysine; me, methyl; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PAG, periaqueductal grey; ph, phosphate; PVN, paraventricular nucleus.

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The authors conclude that besides miR-132, which was found to be affected in three independent studies (Konopka *et al*. 2010, Nudelman *et al*. 2010, Wang *et al*. 2013), findings on other miRNAs were never replicated. Determining the role of miRNAs in regulatory processes remains a major challenge, as miRNAs often have a wide range of direct molecular targets and might indirectly influence the expression of even more genes by altering the levels of transcription factors (Schouten *et al*. 2013). Hence, identifying important target genes for miRNAs often relies on *in silico* target prediction.

Stress-induced epigenetic modification of the HPA axis

While it is clear that there is a myriad of epigenetic modifications occurring after stress exposure (Box 3), those occurring in genes involved in the regulation of the HPA axis are of particular importance. As mentioned before, stress-related psychopathology is associated with HPA axis dysfunction (Tsigos & Chrousos 2002), which has clear clinical relevance; elevated basal cortisol has for example been shown predictive of the risk for depressive episodes (Goodyer *et al*. 2001), whereas successful antidepressant treatment is associated with the resolution of the impaired HPA axis negative feedback (Pariante 2006) by restoring corticosteroid receptor expression in the brain (Pariante & Lightman 2008), which also predicts the patient's long-term clinical outcome (Pariante 2006). In PTSD, low cortisol levels following trauma have been shown predictive of subsequent PTSD symptomatology (McFarlane *et al*. 1997, Delahanty *et al*. 2000, McFarlane 2000, Witteveen *et al*. 2010), whereas elevating these levels reduced PTSD incidence (Schelling *et al*. 1999, Schelling *et al*. 2001, Schelling *et al*. 2003, Zohar *et al*. 2011). Corticosteroid administration prior to trauma was shown to reduce PTSD symptoms (Schelling *et al*. 2004, Weis *et al*. 2006), whereas preliminary work indicated that chronic corticosteroid treatment of PTSD patients reduces symptomatology (Aerni *et al*. 2004). In this section, we will discuss how stress-induced epigenetic alterations in adult life can mediate changes in HPA axis function through affecting CRH and glucocorticoid signalling, mainly in the hypothalamic PVN, hippocampus, and PFC.

Corticotropin-releasing hormone signalling

CRH expression in the PVN, amygdala and bed nucleus of the stria terminalis is related to a wide range of stress-adaptive responses, including the autonomic, immune, and behavioural domain (Kovacs 2013). Stress exposure generally increases PVN *CRH* mRNA and peptide levels, peaking at 30 min post-stress and slowly declining thereafter, as observed in rats by Shepard *et al*. (2005). Increased stress-induced *CRH* transcriptional responses have been linked to both early and adult life trauma

Box 3 Mechanisms for stress-induced epigenetic alterations.
While it has been known for quite some years that stress exposure can induce epigenetic modifications in a variety of genes and brain regions, it is still largely unclear by which molecular pathways these effects are exactly established. Recent studies have however started to elucidate these mechanisms by implicating a novel, non-genomic mechanism by which glucocorticoids act to (amongst others) facilitate consolidation of memories associated with a specific adverse event through epigenetic pathways. Gutierrez-Mecinas *et al*. (2011) observed that binding of glucocorticoids to GRs in rat hippocampal DG granule neurons activated the extracellular signal related kinase (ERK)/mitogen-activated protein kinase (MAPK) signalling pathway. Downstream kinases of this pathway induced serine 10 phosphorylation and lysine 14 acetylation at histone H3 (H3S10p-K14ac) via recruitment of histone acetyl-transferases (Chandramohan *et al*. 2007). This epigenetic mark has been associated with the activation of silent genes, possibly through chromatin remodelling, making them accessible for transcription (Cheung *et al*. 2000, Nowak & Corces 2000). This glucocorticoid-induced H3S10p-K14ac could long-lastingly activate genes that were silent before stress exposure, thereby offering a possible mechanism by which stress could induce stable epigenetic and (eventually) behavioural alterations. Indeed, the interaction of the H3S10p-K14ac mark with the promoter region of the immediate-early genes (IEGs) *c-Fos* and *Egr-1* was found to facilitate the induction of these genes (Gutierrez-Mecinas *et al*. 2011). Injection of a GR-occupying dose of corticosterone in rat hippocampus was however ineffective to form H3S10p-K14ac and induce IEG expression, suggesting the required involvement of another molecular pathway in mediating these effects (Chandramohan *et al*. 2007). The NMDA receptor was later identified as a co-activator of the MAPK pathway, whose synchronised activation is necessary for formation of H3S10p-K14ac and IEG induction (Reul *et al*. 2009). For an extensive review describing this glucocorticoid control over epigenetic modifications, Reul *et al*. (2015).

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exposure (Plotsky & Meaney 1993, Chen *et al*. 2012, Mironova *et al*. 2013, Xu *et al*. 2014, Eraslan *et al*. 2015), and epigenetic mechanisms may underlie these changes. Sterrenburg *et al*. (2011) reported on demethylation of the *Crh* promoter region and subsequent CRH upregulation in the PVN of stressed rats compared to controls as a consequence of 14-day chronic variable mild stress. Similar alterations have been observed in the mouse PVN following chronic social defeat stress in vulnerable vs resilient animals (Elliott *et al*. 2010), demonstrating a direct link between the epigenetic alterations and the observed social avoidant phenotype. DNMT3B and HDAC2 in the PVN were decreased and the demethylation-promoting factor GADD45 was substantially upregulated 1 h after the last social defeat session in defeated vs control animals, suggesting their involvement in *Crh* demethylation. The increased CRH levels, demethylation of *Crh*, and the decrease in HDAC2 remained present for at least 2 weeks after the end of social defeat. CRH is thought to exert its overall anxiogenic effects by binding to CRH receptor 1 (CRHR1) (Henckens *et al*. 2016). A recent study showed that 21 days of CUS decreased hypothalamic H3K9 trimethylation in the rat, which was correlated with elevated levels of local CRHR1 expression and avoidance behaviour (Wan *et al*. 2014). Moreover, Sotnikov *et al*. (2014) showed that amygdalar CRHR1 expression was regulated by *Crhr1* methylation and correlated with trait anxiety, substantiating the link between epigenetic regulation of the CRH-CRHR1 system and the anxious phenotype induced by stress. A growing body of evidence demonstrates that also miRNAs can regulate the expression of HPA axis-related target genes. Haramati *et al*. (2011) reported on decreased levels of amygdalar miR-34c following acute social defeat, which was found to target *Crhr1* via a complementary site on the 3′ untranslated region of the receptor transcript. Overexpression of miR-34c appeared to reduce cell responsiveness to CRH by inhibiting CRHR1 expression and induce an anxiolytic phenotype. Among the predicted targets of the miR-34c family were also other stress-related proteins, including brain-derived neurotrophic factor (BDNF) and 5-HT and glutamate receptors. These data suggest that miR-34c plays a role in regulating multiple amygdalar genes that collectively modulate the behavioural response to stress.

An important modulator of CRH expression is the BDNF. BDNF is able to induce expression of CRH in the PVN by binding to hypothalamic tropomyosin receptor kinase B (TrkB) receptors. TrkB activation induces expression of cAMP response element-binding protein, which binds to the *Crh* promoter region and acts as a transcriptional activator (Jeanneteau *et al*. 2012). BDNF in the rat PVN has been found to be upregulated by chronic restraint stress, concurrent with elevated *Crh* mRNA levels (Naert *et al*. 2011). Upregulation of PVN BDNF by stress-induced epigenetic modifications could therefore contribute to the increased CRH expression and the HPA axis dysfunction that is observed in rodents following chronic stress in adulthood (Zhu *et al*. 2014) and in human stress-related pathology (Tsigos & Chrousos 2002). In contrast, both acute and chronic stress have been found to reduce BDNF expression in the mouse and rat hippocampus, which was associated with increased local H3K27 methylation (Tsankova *et al*. 2006), decreased H3 acetylation (Fuchikami *et al*. 2009), and enhanced *Bdnf* promoter methylation (Roth *et al*. 2011, Niknazar *et al*. 2016). Furthermore, hippocampal expression levels of TrkB were reduced following forced swim stress, which increased methylation of *Trkb* (Niknazar *et al*. 2016). Decreased hippocampal BDNF has been hypothesized to underlie hippocampal dysfunction in response to traumatic stress (Johnsen & Asbjornsen 2008, Moore 2009), as BDNF is an important neurotrophic factor that enhances long-term potentiation and other forms of synaptic plasticity in the hippocampus (Korte *et al*. 1996). Indeed, overexpression of hippocampal BDNF has been found to mediate behavioural resilience to chronic mild stress in rats (Taliaz *et al*. 2011). Despite evidence for altered *Bdnf* methylation levels in rodent PVN and hippocampus, plasma BDNF levels and *BDNF* methylation status were not found to be altered after acute psychosocial stress in healthy human subjects (Unternaehrer *et al*. 2012).

Corticosterone signalling

Glucocorticoid receptor Many of the behavioural effects of stress-induced corticosteroid release are thought to be mediated by activation of GRs (McKlveen *et al*. 2013, Park *et al*. 2015). Moreover, corticosteroid binding to GRs contributes to the negative feedback inhibition of the HPA axis, which is important in the termination of the stress response. This negative feedback loop is disrupted in PTSD, thought to be mediated by increased GR expression levels in the PFC and hippocampus (Mizoguchi *et al*. 2003). This implies that altered regulation of GR transcription by epigenetic modifications serves as a potential underlying mechanism. Demethylation of *NR3C1*, the gene coding for GR, was observed in blood and saliva from PTSD patients vs trauma-matched healthy controls (Labonte *et al*. 2014, Vukojevic *et al*. 2014,

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Yehuda *et al*. 2015). *NR3C1* methylation levels even inversely correlated with PTSD symptom severity, emphasizing its relevance to psychopathology. In contrast, studies in human patients have implicated hypermethylation of *NR3C1* and subsequent decreases in peripheral (Yehuda *et al*. 1993) and cortical (Webster *et al*. 2002) GR levels in the pathogenesis of MDD, suggesting that oppositely directed epigenetic alterations might be responsible for the contrasting HPA axis alterations in PTSD and MDD.

Although these patient studies do not provide evidence for a causal role of trauma exposure to these differences, rodent work has reported on increased DNA hydroxymethylation (5-hmC) of the *Nr3c1* promoter in mouse hippocampus after acute restraint stress exposure (Li *et al*. 2015). Since 5-hmC is associated with active gene transcription (Szulwach *et al*. 2011), these data suggest that the observed increased 5-hmC is likely associated with elevated local GR expression. This would be in line with previous findings that acute stress in adulthood increases hippocampal *Nr3c1* mRNA levels in mice (Gray *et al*. 2014). The study by Li *et al*. (2015) did not detect a stress-related change in total methylation levels (i.e., 5-mC+5-hmC), suggesting that the increase in 5-hmC was paralleled by a decrease in 5-mC, which collectively induced the stress-related NR3C1 upregulation. Stress exposure may additionally induce alterations in the epigenetic regulation of FK506 binding protein 5 (FKBP5), a known regulator of GR sensitivity (Binder 2009), as corticosterone administration during adulthood was shown to increase anxiety-like behaviour and elevate mouse hippocampal FKBP5 expression (and thus potentiate GR sensitivity) by decreasing DNA methylation at the *Fkbp5* locus (Lee *et al*. 2010). These findings collectively suggest that disrupted negative glucocorticoid feedback, as observed in PTSD, is characterized by elevated hippocampal and PFC GR levels, mediated by epigenetic mechanisms on the DNA and RNA level.

In contrast, Uchida *et al*. (2008) reported on the downregulation of GR expression by miRNAs in the rat PVN following repeated restraint stress, a paradigm commonly used to induce a depressive-like phenotype (Gregus *et al*. 2005, Chiba *et al*. 2012). Protein, but not mRNA levels of PVN GR, were found to be significantly lower in repeatedly stressed vs control rats, suggesting the involvement of regulatory mechanisms at the posttranscriptional level. Indeed, miR-18a, targeting two sites of the 3′ untranslated region of *Nr3c1* and downregulating gene expression, was found to be upregulated in the PVN. The finding that GR expression is elevated by acute stressors, but decreased by repeated stressors, might reflect earlier observations that GR expression (and thereby negative feedback regulation) is oppositely affected in MDD and PTSD (Alt *et al*. 2010).

Mineralocorticoid receptor Whereas the role of GR in stress response reactivity and regulation has been extensively studied, the mineralocorticoid receptor (MR), has received less attention. While the GR is associated with regulation of HPA negative feedback and termination of the stress response, the MR, which in humans is encoded by the *NR3C2* gene, is thought to be involved in the appraisal process and onset of the stress response upon binding of glucocorticoids (de Kloet *et al*. 2005). Co-localization of both receptors is found in the hippocampus of almost all species (Patel *et al*. 2000). The receptors collectively orchestrate the stress response as an altered GR/MR balance has been implicated in persistent dysregulation of the HPA axis (Harris *et al*. 2013). As the affinity of the intracellular MR for cortisol and corticosterone is approximately ten times higher than that of the intracellular GR, MRs are already largely occupied even under non-stress conditions (Grossmann *et al*. 2004). Hence, unsurprisingly, the GR has dominated endocrine stress research for a long time. However, a new form of membrane-bound MR was recently shown to exert rapid stress-induced effects on neurotransmission and synaptic plasticity in the hippocampus and amygdala (Joels *et al*. 2008). The apparent affinity of this membrane-located MR is 10-fold lower than that of its intracellular counterpart, demonstrating that the MR might also have a far more prominent role in the behavioural stress response than was previously thought. The possibility of dynamic regulation of MR expression in response to stress has been demonstrated in a preclinical study showing an increase in rat hippocampal MR density after a forced swimming task (Gesing *et al*. 2001), which served to restrain the HPA axis. Hippocampal *Nr3c2* mRNA levels were however found to be decreased by almost 20% due to CUS (Lopez *et al*. 1998), whereas local MR (but not GR) protein levels were reduced following the chronic administration of corticosterone (Wu *et al*. 2013), which was accompanied by depressive-like symptomatology. These results indicate that MR expression is highly responsive to stress exposure, which likely has important consequences for neuroendocrine control of the stress response. *NR3C2* is also subject to epigenetic regulation, but, in contrast to the case of *NR3C1*, only few studies have investigated this. Perroud *et al*. (2014) reported on lower methylation of several CpGs located within the *NR3C2* promoter in trauma-exposed women. While plasma MR levels

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were significantly elevated in these same individuals, no significant correlation was found with the altered *NR3C2* methylation status. Recent findings in rodents (Sober *et al*. 2010) have also implicated miRNAs (miR-135a and miR-124) as potential regulators (i.e., suppressors) of NR3C2 protein expression. An independent study by Mannironi *et al*. (2013) showed that these miRNAs were downregulated in the mouse amygdala following acute restraint stress, which increased amygdalar MR expression.

In conclusion, a growing body of research demonstrates that stress-induced epigenetic alterations underlie a wide variety of aberrations in HPA axis function that are observed in PTSD patients and rodent models of acute and chronic stress. This includes increased CRH expression in the PVN, decreased hippocampal CRH and MR levels, and elevated hippocampal and prefrontal GR expression. The interesting finding that epigenetic regulation of paraventricular GR expression was oppositely affected by acute (Li *et al*. 2015) and repeated (Uchida *et al*. 2008) restraint stress underlines that there is still a knowledge gap pertaining the differential epigenetic profiles of genes involved in HPA axis regulation that underlie PTSD-like and depressive-like phenotypes. This calls for a more structured investigation of the distinct epigenetic changes induced by severe, acute (which induces PTSD-like symptomatology) and chronic stress (known to induce a depressive-live behavioural phenotype).

Stress-related epigenetic modification of stress-related neurotransmitters

Besides modulating the neuroendocrine response to stress, epigenetic modifications may alter neurotransmitter release and signalling in brain circuits that orchestrate the stress response and are known to be altered in PTSD (Southwick *et al*. 1999). Alterations in dopamine (Yehuda *et al*. 1992), norepinephrine (NE) (Geracioti *et al*. 2001), and serotonin (5-HT) (Arora *et al*. 1993) transmission are thought to contribute to the symptoms commonly observed in PTSD patients, including hypervigilance, impulsivity, exaggerated startle, and depressed mood, and may be subject to epigenetic regulation. For example, levels of the enzymes tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH), responsible for creating precursor metabolites for the production of dopamine (DA), epinephrine, NE, and 5-HT, were found to be significantly decreased in the hippocampus of chronically stressed rats (Liu *et al*. 2014), concurrent with decreased hippocampal acetylation of H3K9 and H4K12 and increased levels of HDAC5. Administration of the HDAC inhibitor sodium valproate not only prevented the decrease of H4 acetylation and increase of HDAC5 protein expression, but also blunted the decrease in TH and TPH expression, implicating epigenetic regulation of neurotransmitter precursor production. Direct evidence for altered epigenetic regulation of stress-related neurotransmitters as a consequence of adult life stress exposure primarily exists for the serotonergic system. The serotonin transporter (5-HTT), which in humans is encoded by *SLC6A4*, is an integral membrane protein in the central and peripheral nervous system that transports 5-HT back from the synaptic cleft into the pre-synaptic neuron, thereby waning serotonergic transmission. Reduced expression of this transporter incites high basal 5-HT levels, which has been associated with enhanced vulnerability to chronic stress (Bartolomucci *et al*. 2010) and increased risk for life time depression (Kambeitz & Howes 2015). Resilience to clinical depression under chronic high stress conditions was however found to be associated with reduced methylation of the *SLC6A4* promoter (Alasaari *et al*. 2012), which is expected to increase 5-HTT expression (Philibert *et al*. 2008). Increased reuptake of 5-HT by 5-HTT and subsequent basal 5-HT decrease might therefore be a mechanism of stress adaptation, contributing to chronic stress resilience. Chronic stress was also found to induce a long-lasting upregulation of serotonin receptor 1A (5-HT1A) RNA and protein levels in the mouse mPFC and dorsal raphe nucleus (Le Francois *et al*. 2015), corroborating the evidence of altered epigenetic regulation of serotonergic transmission as a consequence of adult life stress exposure. This stress-induced increase in *5-Ht1a* mRNA was paralleled by the increased methylation of a uniquely conserved CpG site in *5-Ht1a* that serves as a binding site for the transcriptional repressor Sp4, explaining the observed upregulation in expression. Yet, it is unknown how these changes in 5-HT1A expression affect serotonergic transmission, as they may upregulate 5-HT1A in different cell (interneurons vs pyramidal cells) and receptor types (post-synaptic receptors vs autoreceptors) which regulate serotonergic network activity in an opposite manner.

Findings from human studies have indicated that epigenetic modifications, besides having a direct modulatory effect, can also interact with the genotype to shape the stress response. DNA methylation profiles within *SCL6A4* were found to moderate the association of the 5-HTT linked polymorphic region (5-HTTLPR) and stress coping (van IJzendoorn *et al*. 2010, Alexander *et al*. 2014). High serum *SCL6A4* methylation was associated with an increased risk of unresolved responses to loss or

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other trauma in carriers of the usually protective 5-HTTLPR long allelic variant, while low levels of methylated *SCL6A4* predicted unresolved loss or trauma in short allele carriers.

Conclusion and future directions

In this review, we have provided a comprehensive overview of several lines of evidence suggesting that epigenetic modifications form an important link between stress exposure in adult life and the resulting persistent changes in gene expression and behaviour associated with stress-related psychopathology. This epigenetic regulation can be found at the level of many mediators of the stress response, including neuroendocrine components of the HPA axis and stress-related neurotransmitter system. Epigenetic mechanisms have been shown to underlie the stress-induced alterations in the HPA axis that are observed in PTSD patients and rodent models of acute and chronic stress. This includes increased CRH expression in the PVN, decreased hippocampal CRH and MR levels, and elevated hippocampal and prefrontal GR expression. This knowledge can be of critical importance to treat stress-related symptomatology.

While the reviewed rodent studies provide valuable insights into the relatively short-term epigenetic response to adult life stress, a thorough assessment of persistent epigenetic changes over prolonged periods of time is required to better model the lasting and intrusive nature of stress and trauma exposure on neuroendocrine function and the associated neuropsychiatric symptomatology. Almost all studies into acute and chronic stress investigated 'snapshot' epigenetic marks, assessed at one time point and relatively shortly (1–28 days) post-stress exposure. It would, however, be interesting to (i) test for the involvement of epigenetic mechanisms in the long-lasting behavioural effects of transient stress exposure and (ii) to assess whether it is possible to distinguish timeframes in which particular stress-induced epigenetic programming takes place and pose opportunity windows for treatment. Long-term research is already being performed to study the epigenetic consequences of early life stress during adulthood, for example by Bockmuhl *et al*. (2015) and Pusalkar *et al*. (2016), who followed up rats and mice for 6 and 15 months after perinatal stress, respectively. Applying similar study designs to follow up rodents for several months after adult life stress induction could yield valuable information about the epigenetic processes and marks that play a role in the induction of long-term depressive and anxious phenotypes by stress experienced in adulthood. Furthermore, to establish whether particular epigenetic programming occurs in specific timeframes, it would be of utmost importance to assess brain epigenetic marks at multiple time points following stress induction. However, invasive measurements of the brain can only be performed once, reiterating the importance of including non-brain-based epigenetic biomarkers such as blood. Previous findings from methylome-wide profiling have indicated that around 50% of differentially methylated positions in rat hippocampus and cortex are mirrored in the blood (Davies *et al*. 2012, Aberg *et al*. 2013), affirming that findings in the blood may be have great value as a proxy for brain tissue. Once important epigenetic markers in brain tissue are identified (especially in the case of very specific modifications of a single gene) one could test to see if these markers are also present in blood or saliva samples. If so, the experiment can be repeated to longitudinally measure this biomarker at multiple time points post-stress to assess the longevity of the marker and its correlation to long-lasting stress-induced behaviour.

While evidence is accumulating for a crucial role of epigenetic modifications in the pathology of stress-related disorders, the next step should be to apply this knowledge to prevent and treat these disorders by targeted interventions. Once we have an overview of the maladaptive epigenetic changes that occur after stress exposure that are linked to neuropathology; is it possible to revert these changes and to remodel the stress-vulnerable brain to a stress-resilient brain? Although there is clearly a window of increased plasticity for epigenetic programming during early life, the stress-induced epigenetic changes occurring as a consequence of stress during early life and adulthood are likely similar and could therefore be reverted by employing similar strategies. Initial studies have already reported on successful treatment strategies that are not conceptually different from those that are also being used to reprogram the epigenetic effects of early life stress. Preliminary findings in adult rodents have focused on five possible intervention/treatment strategies:

(i) Antidepressants. The tricyclic antidepressant imipramine and the selective serotonin reuptake inhibitor fluoxetine have been shown to revert stress-induced histone demethylation (Hunter *et al*. 2009) and methylation (Wilkinson *et al*. 2009), demethylation of *Crh* (Elliott *et al*. 2010), methylation of *Bdnf* (Tsankova *et al*. 2006) and *5-Ht1a* (Le Francois *et al*. 2015) and decreased levels of HDAC5 (Renthal *et al*. 2007), which all reduced depressive and anxiety-like behaviour induced by the respective stress protocols.

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(ii) HDAC inhibitors. The HDAC inhibitors sodium valproate and MS-275 have been shown to reduce depressive and anxiety-like behaviour by reverting stress-induced increases in HDAC2 and HDAC5 and subsequent histone acetylation marks on H3K9, H3K14 and H4K12 (Covington *et al*. 2009, Liu *et al*. 2014).

(iii) DNMT inhibitors. The DNMT inhibitor RG108 has been shown to reduce depressive and anxiety-like behaviour by reverting stress-induced increases in DNMT3A (Elliott *et al*. 2016).

(iv) miRNAs. The amygdalar miRNA-34 has been identified as a repressor of stress-induced anxiety (Haramati *et al*. 2011). As such, miRNA-34 and other stress-related miRNAs pose potential novel targets for treatment of stress-related disorders.

(v) Exercise. Physical exercise has been shown to improve cognitive responses to psychosocial stress and rescue rats from social defeat-induced anxiety-like behaviour and memory impairment (Collins *et al*. 2009). This beneficial effect might potentially be mediated by epigenetic mechanisms, including exercise-induced H3 acetylation and modulation of methylation in the hippocampus (Patki *et al*. 2014).

However, it is currently mechanistically unclear whether the behaviourally beneficial effects of these treatments are mediated directly through an effect on the epigenome, or through another external mediator affecting both behaviour and epigenetic markers independently. Because these treatment strategies all have a broad scope and potentially affect a wide range of processes in the body, higher precision epigenetic editing might be necessary to specifically target epigenetic marks in the brain and enable personalized medicine. Still, these preliminary results show that it possible to attend to the behavioural consequences of stress exposure by pharmacological and therapeutic interventions targeting epigenetic profiles.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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