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Neuroepigenetics of Post-traumatic Stress Disorder

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Abstract

While diagnosis of PTSD is based on behavioral symptom clusters that are most directly associated with brain function, epigenetic studies of PTSD in humans to date have been limited to peripheral tissues. Animal models of PTSD have been key for understanding the epigenetic alterations in the brain most directly relevant to endophenotypes of PTSD, in particular those pertaining to fear memory and stress response. This chapter provides an overview of neuroepigenetic studies based on animal models of PTSD, with an emphasis on the effect of stress on fear memory. Where relevant, we also describe human-based studies with relevance to neuroepigenetic insights gleaned from animal work and suggest promising directions for future studies of PTSD neuroepigenetics in living humans that combines peripheral epigenetic measures with measures of central nervous system activity, structure and function.

Keywords

Fear memory; stress; animal models; DNA cytosine modifications; histone modifications; HDAC inhibitors

Introduction

Post-traumatic stress disorder (PTSD) is a psychiatric disorder that presents with intrusive and persistent re-experiencing of traumatic events, avoidance of distressing trauma-related stimuli, negative alterations in cognition and mood, and alterations in arousal/reactivity after exposure to a traumatic event¹. While most people experience a traumatic event at some point in their lives (~50–90% of US population^{2–4}, ~70% world-wide^{5,6}), only a small

subset develop PTSD over a lifetime (~7% and ~4% lifetime prevalence of PTSD in the US^{7,8} and across countries⁵, respectively). This suggests that exposure to trauma is necessary, but not sufficient, for the development of PTSD, and that pre-existing factors, including genetic background and early life experiences, interact with trauma exposure, via epigenetic mechanisms, to elicit a spectrum of individual responses to trauma.

Epigenetic mechanisms biochemically encode individual experiences and environmental exposures into the genomic architecture of each cell to modulate gene expression, and, by extension, cell functioning and phenotype. In this way, the epigenome provides the genome with constant regulatory feedback from environmental cues and serves as a link between pre-existing factors and response to trauma exposure. Since PTSD requires exposure to a traumatic event as a diagnostic criterion, it serves as an ideal model for distinguishing between predisposing vulnerability/resilience factors and response to precipitating trauma exposure, which may be encoded as short-lived or persistent epigenetic changes that propagate posttraumatic physiological changes and symptoms.

While diagnosis of PTSD is based on behavioral symptom clusters that are most directly associated with brain function, epigenetic studies of PTSD in humans (detailed in these reviews^{9,10}) have been limited to peripheral tissues, such as blood, buccal tissue, and saliva. Very few studies have been conducted in postmortem human brain tissues of individuals with PTSD^{11–14}, and none have explored epigenetic mechanisms. Animal models of PTSD (reviewed here^{15–17}), predominantly in rodents, have been key for understanding the epigenetic alterations in the brain most directly relevant to endophenotypes of PTSD, which can broadly be divided into dysregulation of fear memory and stress response. In this chapter, we will discuss neuroepigenetic studies based on animal models of PTSD, with an emphasis on the effect of stress on fear memory. Where possible, we will relate these findings to those from the human PTSD literature, conducted in peripheral tissue, and suggest promising directions for future studies of PTSD neuroepigenetics in humans.

Neuroepigenetic regulation of fear learning and memory

One of the hallmark symptoms of PTSD is the persistence of intense fear memories that underlie intrusive re-experiencing of traumatic events. Recall of these traumatic memories may be triggered by non-threatening stimuli under safe conditions due to inappropriate generalization of trauma-associated stimuli^{18,19}, and is thought to reflect impaired fear extinction learning and overactive fear memory consolidation and reconsolidation²⁰. Animal models of PTSD examining the neural underpinnings of fear learning and memory have utilized fear conditioning paradigms^{20,21}, and have been instrumental for investigating the global and gene-specific neuroepigenetic mechanisms underlying each stage of fear memory processing. These studies have focused on key brain regions dysregulated in PTSD, notably the amygdala, hippocampus, and medial prefrontal cortex (mPFC)^{22,23}, and have provided many insights into the dynamic regulation of participating epigenetic processes, which themselves direct the transcriptional and translational activities involved in synaptic plasticity, fear memory processing, and behavioral response.

While the fear conditioning paradigm and fear circuitry (Fig. 1) have been reviewed elsewhere^{20,21,24–26}, an outline of the different stages of fear memory sets the framework for the current discussion. Memory formation involves stabilization of the acquired memory (i.e., memory consolidation) and requires *de novo* protein synthesis, with contextual processing and associations highly dependent on the hippocampus and cue associations dependent on the amygdala^{26,27}. Recently consolidated contextual fear memories in the hippocampus are transferred to the cortex for long-term, remote memory storage and maintenance (i.e., systems-level memory consolidation)²⁸, while long-term storage of cued fear associations remain localized in the amygdala²⁹. Memory retrieval destabilizes previously consolidated memories and requires reconsolidation to re-stabilize, and possibly update, these labile memory traces^{30,31}. By presenting fear-associated conditioned stimuli without the fear-inducing unconditioned stimulus, new extinction memories can be learned on top of the original fear associations. Taken together, memories are dynamic and can be transformed over time via strengthening/weakening of memory traces or via updating/overriding of traces by integration of new experiences³⁰. Understanding these processes will enable development of preventative interventions and treatments for PTSD that combine medication and cognitive-behavioral therapies and inform effective delivery of interventions.

Molecular mechanisms of fear memory reconsolidation and extinction

The persistent and intrusive recall of traumatic memories in PTSD is most directly associated with dysregulated enhancement of fear memory reconsolidation and impairment of fear memory extinction²⁰. Therefore, PTSD-relevant interventions and preclinical evaluations have focused on interference of reconsolidation, to weaken or erase the original traumatic memory (i.e., forgetting), and on strengthening of extinction learning, to learn “disassociation” of fear-inducing component of memory while leaving the rest intact^{32,33}.

While a distinct process, reconsolidation shares many key features with consolidation, such as activation of extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) signaling^{34,35}, which regulates histone modifications, and interaction of different neuroepigenetic mechanisms³⁶. For example, retrieval of fear memory was found to promote global increase in histone H3 acetylation and phosphorylation in the CA1 region of the hippocampus³⁷, and in histone H3 acetylation in the lateral amygdala (LA; phosphorylation was not examined in the latter study)³⁸, thus inducing increased transcriptional activity in these brain regions. Additionally, histone hyperacetylation in the LA, induced via histone deacetylase (HDAC) inhibitors shortly after retrieval of fear memory, was shown to enhance reconsolidation of cued fear memories; in contrast, histone hypoacetylation, induced via infusion of either broad spectrum or p300/CBP-specific histone acetyltransferase (HAT) inhibitors showed the opposite effect^{39,40}. Since improvement of memory consolidation with HDAC inhibitors has been associated with enhanced induction of long-term potentiation (LTP)^{41,42} and dendritic sprouting⁴³ similar mechanisms may be involved in fear memory reconsolidation. Similarly, inhibition of DNA methyltransferase (DNMT) shortly after retrieval impairs long-term reconsolidation of cued fear memory in the LA⁴⁴. As observed in consolidation, pretreatment with an HDAC inhibitor was able to rescue deficits due to DNMT inhibition³⁸, illustrating a key role for the interaction between epigenetic processes in the regulation of fear memory and synaptic plasticity.

Fear extinction learning is also associated with increased levels of histone acetylation⁴⁵; while fear extinction formation and consolidation is highly dependent on the infralimbic region of the ventromedial prefrontal cortex (ILPFC)⁴⁶, systemic, intrahippocampal and intra-ILPFC infusion of general HDAC inhibitors, before and after contextual fear memory retrieval, have all been shown to enhance extinction learning^{45,47}. Additionally, HDAC inhibitor treatments have been shown to increase histone acetylation in association with fear extinction enacted with weaker extinction paradigms, which otherwise would not have been successful,^{45,48} with corresponding effects on mRNA expression⁴⁸. Together with the reconsolidation-based findings described above, these fear extinction-related findings demonstrate that retrieval and long-term consolidation of fear memories, as well as fear extinction learning, involve multiple, potentially interacting epigenetic processes that can impact downstream gene function.

Another factor with effects that are epigenetically regulated is time since initial fear memory formation. While retrieval of recent memories (i.e., 1 day) induces a limited period of hippocampal neuroplasticity, such plasticity is absent or reduced in retrieval of remote memories (i.e., 1 month), and corresponds to differences in fear extinction training success and epigenetic patterns. This time-related difference in memory lability is particularly relevant to PTSD, as the disorder requires persistence of symptoms for at least one month for a diagnosis. More specifically, relative to remote memory retrieval, recent memory retrieval induces more abundant H3K9/14 acetylation and lacks HDAC2 binding at the promoter of *Fos*, an immediate early gene (IEG) crucial for neuronal activation, and is more successful in fear extinction learning (i.e., reconsolidation updating)⁴⁹. This difference in HDAC2 binding was determined to be mediated by an increase in HDAC2 nitrosylation (which releases HDAC2 from chromatin⁵⁰) after recent, but not remote, memory retrieval⁴⁹. Importantly, remote memories were persistently attenuated (i.e., successfully extinguished) following application of an HDAC2-targeting inhibitor, which was able to “epigenetically prime” the expression of neuroplasticity-related genes⁴⁹ - suggesting potential capability for inducing neuroplasticity in remote memories. These animal-based findings resolve the effects of various factors, such as type of epigenetic-modifying adjuvant, mainly HDAC inhibitors, specific paradigm for extinction-based exposure therapy, timing of adjuvant delivery during therapy, and time since original trauma memory, and have translational implications for informing treatment planning that is optimized and tailored for individual cases.

Dynamic regulation of DNA cytosine modifications is also implicated in fear extinction learning, and studies suggest that hydroxylation of 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) in the ILPFC is important for successful extinction^{51,52}. Li et al.⁵² revealed that fear extinction leads to rapid Tet3-mediated, global 5hmC redistribution that may drive selective accumulation of 5hmC for promoting a “primed” transcriptionally active epigenetic state in the ILPFC. Similarly, Rudenko et al.⁵¹ demonstrated the importance of Tet1 for fear memory extinction using Tet1 knockout (KO) mice. Impairment of extinction learning in these mice was associated with hypermethylation of the *Npas4* promoter region after extinction training, which has implications for the regulation of many neuronal activity-regulated genes, including *Fos*⁵¹. A recent study examining how epigenetic marks coordinate regulation after memory retrieval reported an important link between

H3K4me3 and 5hmC marks in the hippocampal CA1 region and anterior cingulate cortex (ACC): Retrieval of a recent, contextual fear memory increased H3K4me3 and 5hmC levels, both globally and at a CpG-enriched coding region of *Npas4*—but not *Fos*—in the hippocampal CA1 region⁵³. In contrast, retrieval of a remote memory increased H3K4me3 and 5hmC levels at CpG-enriched coding region of *Fos*—but not *Npas4*—in the ACC⁵³. These findings reveal important insights regarding how epigenetic marks coordinate differential targeting of important genes based on brain region and time since initial fear memory formation.

A key gene that was previously noted to play a pivotal role in fear memory formation has also been found to be critical for fear extinction, with supporting evidence in humans. Specifically, Soliman et al.⁵⁴ revealed that the brain-derived neurotrophic factor (*BDNF*) Val66Met genotype (*BDNF*_{Met} variant) was associated with impaired fear extinction learning in both humans and an inbred genetic knock-in mouse strain expressing a variant *BDNF* allele. This is further supported in the human literature by analyses revealing that *BDNF* Val66Met genotype predicts response to exposure therapy in PTSD⁵⁵, and in the animal literature by similar reports of impairment in extinction learning using a conditioned taste aversion paradigm on the *BDNF* variant knock-in mouse strain⁵⁶. In the epigenetic literature, fear extinction was shown to increase histone H4 acetylation at *Bdnf* promoter 4 and mRNA levels of *Bdnf* exon I and IV in the PFC⁴⁸. Of note, BDNF signaling may be involved in the sex differences observed in fear extinction learning. Baker-Andresen et al. (2013) found that female mice exhibited impaired retention of fear extinction and greater DNA methylation increase, with corresponding mRNA level decrease, of *Bdnf* exon IV in the medial PFC, compared to male mice⁵⁷. They further explored targeted activation of BDNF signaling in female mice by administering a TrkB agonist before memory retrieval and extinction training and found that this sexually dimorphic impairment in fear extinction learning and fear response was blocked⁵⁷. (However, it was not tested in male mice.) This finding has translational implications for potential sex-specific interventions in PTSD.

In addition to DNA cytosine and histone modifications, non-coding RNAs, especially microRNAs (miRNA), are emerging as key epigenetic players. While the role of miRNA in reconsolidation has not yet been reported, a number of studies implicate miRNA regulation in fear extinction. Fear extinction training leads to an activity-dependent increase in miR-128b expression in the ILPFC, and is proposed to facilitate extinction by negatively regulating genes associated with plasticity and retrieval of original fear memory, such as *Rcs*⁵⁸. Additionally, overexpression of miR-144–3p in the BLA has been shown to rescue fear extinction memory in extinction-impaired mice and is thought to target plasticity-associated genes⁵⁹. Of note, *Pten*, which regulates plasticity-associated signaling cascades, colocalizes with miR-144–3p in BLA and shows functional downregulation following successful fear extinction⁵⁹. In humans, blood *DICER1* expression was found to be significantly lower in PTSD with comorbid depression, implicating dysregulation of miRNA processing⁶⁰. Consistent with this, lower blood *DICER1* expression was also significantly associated with higher amygdalar activation to fearful stimuli – a neural intermediate phenotype for PTSD⁶⁰ and depletion of *DICER1* in the central amygdala was demonstrated to increase anxiety-like behavior in mice via inactivation of miRNA processing⁶¹. These

studies suggest that miRNAs serve an important role in the regulation of fear extinction and stress response.

Effect of stress on fear learning and memory

Traumatic events imprint particularly strong memories compared to other life events and may also influence future memory formation of related events. This is reflected in investigations that have revealed that stress exposure prior to fear conditioning enhances conditional fear responding and impairs fear extinction (as outlined in^{62–64}; Fig. 2). This stress-induced sensitization of fear response is largely mediated by the hypothalamic-pituitary adrenal (HPA) axis, which signals systemic release of glucocorticoids (GC) and glucocorticoid receptor (GR) activation, for regulation of GC-responsive genes and other transcription factors. GRs, encoded by the *NR3C1* gene, are found throughout the brain and are particularly abundant in the hippocampus, where they influence contextual memory formation and play a major role in coordinating behavioral, autonomic, and neuroendocrine facets of stress response^{64,65}.

Stress-induced GC binding to GRs has been shown to strengthen memory consolidation of stressful events and induce epigenetic alterations, globally⁶⁶ and specifically to activate expression of neuroplasticity-related genes⁶⁷. This has been demonstrated both in studies that utilize fear-conditioning paradigms, which are inherently stressful, as well as various stress paradigms. Psychological stressors have been shown to significantly increase the number of granule neurons stained for histone phosphorylation and acetylation (i.e., H3S10p-K14ac) in the dentate gyrus (DG) of the hippocampus, and this increase can be inhibited by GR antagonists⁶⁸. Additionally, these histone marks were found to specifically associate with promoters of *Fos* and *Egr1/Zif268/Ngfia*, another IEG crucial for fear learning and behavior-activated neuronal activity^{69,70}, thereby facilitating a rapid induction of gene expression. Signaling pathway studies have revealed that stress-induced GR activation can facilitate rapid NMDAR-mediated ERK-MAPK signaling to downstream nuclear kinases MSK1/2 and Elk-1, for induction of these transcriptionally activating histone marks in the DG⁶⁴. These findings underscore the GR-dependent epigenetic mechanisms required to mediate gene expression within the DG following a psychologically stressful challenge, and shed light on the molecular substrates for inducing long-term changes in neuronal function linked to memory consolidation of stressful experiences⁶⁴,

From a locus-specific perspective, *Bdnf* has received considerable interest as a mediator of stress-sensitized fear response, as it has with the fear extinction studies described above. *Bdnf* is transcriptionally regulated by GR in an exon- and tissue-specific manner and is differentially expressed in response to stress⁷¹. In addition to fear conditioning studies, which revealed alterations in regulation of *Bdnf* expression via various histone modifications and DNA methylation marks, Roth et al.⁷² used a complex psychosocial stress paradigm (involving restraint stress, exposure to a live predator, and social housing instability) to induce hypermethylation of the *Bdnf* exon IV promoter in the dorsal DG and, more prominently, in the dorsal CA1 region of the hippocampus, with corresponding decrease in *Bdnf* mRNA levels in these regions. Interestingly, they also reported hypomethylation of *Bdnf* exon IV in the ventral CA3 and no methylation changes in other hippocampal

subregions, suggesting that psychosocial stressors selectively drive DNA methylation dynamics, particularly on the *Bdnf* gene, in key dorsal hippocampal regions and may underlie the persistent deficits in memory and stress regulation observed in PTSD⁷². The CA1 region-specific down-regulation of *Bdnf* was also noted in another frequently used model of PTSD¹⁵, which exposed rats to a predator scent stressor (PSS) and differentiated individuals that exhibited a PTSD-like “extreme behavioral response” (EBR) from those with “minimal behavioral response” (MBR)⁷³. The consistency of findings across these different models of PTSD provides robust evidence for the involvement of hippocampus-specific *Bdnf* dysregulation in the pathophysiology of PTSD. This region-specific epigenetic difference may also underlie dysregulation of the fear circuitry by producing an imbalance of BDNF production between the hippocampus and amygdala⁷⁴.

While classical fear conditioning provides the foundation for understanding fear memories and is inherently stressful, animal models of PTSD that expose the animal to more intense trauma/stressor(s) are better able to reproduce core PTSD symptoms^{15,16}. A variety of environmental, physical, and psychological stressors exist for animal models of PTSD, and some well-validated models include single prolonged stress (SPS)⁷⁵ and its adaptations⁷⁶, predator scent stress (PSS)¹⁵, and predator-based psychosocial stress paradigms⁷⁴. Animals exposed to these stressors exhibit characteristics that reflect those generally observed in PTSD, such as increased anxiety-like behaviors, exaggerated startle, and abnormal GC response (i.e., reduced basal GC levels and enhanced GC negative feedback), indicating that they may be appropriate for investigating psychological, trauma-induced cognitive/behavioral and neuroendocrine alterations^{17,77,78}. In particular, animal models of PTSD that expose animals to trauma/stressor(s) before fear conditioning show greater face, construct, and predictive validity for PTSD than fear conditioning alone^{62,75}, and can be used to determine the epigenetic effect of stress on subsequent fear learning⁶³. In conjunction with our understanding from studies that investigated neuroepigenetics of stress and fear memory independently, epigenetic investigations using paradigms such as stress-enhanced fear learning (SEFL) should elucidate distinct mechanisms underlying the interaction between these two major facets of PTSD and have special promise for identifying mechanisms underlying resistance to extinction learning and the role of the BDNF pathway, which is widely implicated in both stress and fear memory⁶³.

While only a few epigenetic studies have been based on the SEFL model so far, their findings are generally consistent with and contribute to our current understanding of the neuroepigenetics behind stress and fear learning. Takei et al.⁷⁹ used a SEFL paradigm with a single prolonged stress (SPS) before contextual fear conditioning to show that SPS rats had higher levels of contextual freezing than sham-treated rats. SPS rats also showed increased acetylation of histone H3 and H4 at the exon I and IV *Bdnf* promoter regions that corresponded with increased exon I and IV *Bdnf* mRNA and total BDNF protein levels in the hippocampus after fear learning, relative to sham treated rats⁷⁹. Additionally, protein levels of BDNF receptor TrkB were significantly higher in hippocampus of SPS rats compared to controls, suggesting enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in this animal model of PTSD⁷⁹. Furthermore, subsequent work showed that treatment with an HDAC inhibitor using this SPS SEFL paradigm facilitated contextual

fear extinction in the hippocampus, and was accompanied by an increase in acetylated histones H3 and H4 in this brain region⁸⁰.

Other studies have revealed significant SEFL-induced alterations in DNA methylation levels on key anxiety- and stress-related genes, including the anxiety-related neurotensin 1 receptor gene, *Ntsr1*, and local GR regulator, *Fkbp5*, in the amygdala. Toda et al.⁸¹ applied maternal separation as a stressor to find increased DNA methylation levels in the promoter region of *Ntsr1*, which corresponded with decreased *Ntsr1* mRNA levels⁸¹. Sawamura et al.⁸² used the restraint/immobilization stressor to demonstrate that dexamethasone treatment (i.e., HPA axis suppression) prior to extinction training enhances stress-sensitized fear extinction learning and corresponds with alterations in *Dnmt/Tet* and *Fkbp5* DNA methylation levels that regulate expression of these genes in the amygdala⁸². These findings suggest that dexamethasone treatment enhances fear extinction learning by inducing dynamic regulation of DNA methylation to alter *Fkbp5*-mediated glucocorticoid (GC) sensitivity.

In addition to epigenetic regulation of specific genes of interest, Qingzhen et al.⁸³ found distinct long non-coding RNA (lncRNA) expression profiles associated with SEFL-PTSD-like syndrome in the hippocampus, and while our understanding of lncRNAs is still scarce, this study suggests a possible role for lncRNA in PTSD dysregulation, a finding that echoes at least one genome-based study of PTSD in humans⁸⁴. When looking at specific lncRNA and pathways, they reported alterations in lncRNA associated with regulating *Fos* and *Camk2a*⁸³, both of which have been implicated in learning and memory processes in previous works^{85,86}. Another expression study using the SEFL paradigm found that PTSD-like mice exhibit a general transcriptional attenuation profile that is associated with upregulation of HDAC5 (but no changes in DNMTs or HDAC2) in the bed nucleus of the stria terminalis (BNST), an important brain region involved in integration of stress/fear response, suggesting a role for this specific histone deacetylase enzyme in regulating PTSD-associated expression in the BNST⁸⁷. Taken together, these studies demonstrate that SEFL phenotypes are likely regulated by multiple epigenetic mechanisms, and suggest that interventions targeting these mechanisms hold promise for treatment of PTSD⁸⁰.

Epigenetic effects of stress/trauma response in the brain and periphery

PTSD is most frequently an acute stress response to trauma exposure that fails to return to homeostasis and further progresses after onset, into continued acute stress response to recurring aversive memories of the traumatic event(s), and chronic or repetitive stress response stemming from PTSD symptoms themselves⁸⁸. Physiologically, this distress can be characterized in stages of systemic dysregulation⁸⁹, including anatomical sites outside of the brain, which may be partially coordinated by maladaptive alterations of hypothalamic-pituitary adrenal (HPA) axis and sympathetic nervous system (SNS) sensitivity/responsivity, as well as neuroimmune dynamics^{88,90}. Although an increasing number of human-based epigenetic studies of PTSD have adopted an epigenome-wide association study (EWAS) approach (e.g.^{91–98}), the bulk of studies published to date have focused on candidate genes within these and related systems (Fig. 3). A number of comprehensive reviews have been published in recent years, and the reader is referred to this work for more detailed

information on these studies^{10,99–101}. Here, we focus our discussion on human-based studies with relevance to the neuroepigenetic insights gleaned from animal work.

Human-based epigenetic studies involving fear learning and memory

As outlined in the earlier sections of this chapter, consolidation/reconsolidation and extinction of fear memories play a key role in the initial etiology of PTSD, and animal models have contributed much to enhance our understanding of the neuroepigenetic mechanisms involved in these processes. To date, however, there have been only a handful of studies in human clinical populations that parallel in some way the findings from the more mechanistic animal work; importantly, unlike the animal work, these human-based studies have drawn exclusively from living individuals and thus have relied upon measurements of epigenetic factors in peripheral tissues, predominantly whole blood and saliva.

The earliest work to evaluate fear-related behavior in relation to epigenetic factors involved assessing fear inhibition, an intermediate phenotype highly relevant to PTSD risk. Specifically, Norrholm and colleagues¹⁰² sought to test the potential interaction between molecular variation (both genetic and epigenetic) in the catechol-*O*-methyltransferase (*COMT*) locus and PTSD diagnosis on fear-potentiated startle responses during fear conditioning and extinction in a highly traumatized sample drawn from the Grady Trauma Project¹⁰³. Using a well-established model of fear-potentiated startle that measures subjects' ability to discriminate between danger and safety signals¹⁰⁴, the authors found that individuals with higher blood-derived DNA methylation levels at two CpG sites in the promoter region of *COMT* showed higher levels of fear-potentiated startle when presented with the safety signal, compared to individuals with lower DNA methylation at these same two sites. Of note, although the authors reported an association between homozygous Met158 *COMT* genotype and impaired fear inhibition, as well as an association between the same genotype and increased DNA methylation levels at four promoter-region CpG sites, the two CpGs mentioned above were independently associated with impaired fear inhibition, suggesting that epigenetic regulation of this locus may contribute to fear processing in humans.

Additional work evaluating epigenetic associations with fear memory-related phenotypes has involved survivors of the Rwandan genocide¹⁰⁵. In this study, the authors focused specifically on the gene encoding the glucocorticoid receptor (GR), *NR3C1*. The GR binds to cortisol and thus plays a key role in modulating the body's response to stress; in addition, evidence from animal and human studies indicates that elevated glucocorticoids, such as cortisol, both enhance consolidation of emotional memories/fear extinction processes and reduce memory retrieval¹⁰⁶, rendering the study of *NR3C1* particularly salient for understanding PTSD-related epigenetic regulation. Using saliva-derived DNA, the authors found that DNA methylation in a CpG overlapping a transcription-factor binding site was negatively correlated with severity of symptoms related to intrusive memories in male, but not female, survivors of the Rwandan genocide; no associations were found in relation to avoidance and hyperarousal symptoms. Moreover, higher DNA methylation levels were associated with a lower lifetime PTSD risk in men, but not women. Extending these findings with an fMRI study in healthy Swiss subjects, the authors found that increased DNA

methylation at the same *NR3C1* CpG site in men was associated with reduced recognition of previously viewed pictures; and that this higher methylation was associated with increased activation in the ventrolateral prefrontal cortex, a brain region implicated in memory retrieval¹⁰⁷. Intriguingly, in a small study of male combat veterans with PTSD, Yehuda and colleagues found that DNA methylation at the *NR3C1* promoter region, measured in blood, predicted response to prolonged exposure therapy—a therapy grounded in confronting and working through traumatic memories—such that subjects with higher DNA methylation pre-treatment predicted lower self-reported PTSD symptoms at follow-up.¹⁰⁸ Taken together, these findings suggest that higher DNA methylation at functionally relevant CpG sites within the *NR3C1* promoter region may confer a protective effect against vulnerability to PTSD, at least among males. More broadly, these findings suggest that DNA methylation in the GR may index memory-related phenotypes with strong relevance for PTSD, even when measured in peripheral tissues.

Most recently, the first longitudinal EWAS study has identified two replicable differentially methylated regions in *ZFP57* and *RNF39* that become hypomethylated in blood as a response to combat trauma in soldiers who develop PTSD symptoms⁹⁴. These genes have previously been implicated in behavioral susceptibility to stress in animal models. *ZFP57* is involved in transcriptional regulation of genomic imprinting¹⁰⁹ and may play a role in modulating responses to stress in hippocampal neurons through interactions with other transcriptional regulators¹¹⁰. *RNF39* is thought to be involved in the early phase of synaptic plasticity and its expression is upregulated after hippocampal LTP induction^{111,112}. It is also strongly upregulated in the lateral amygdala after Pavlovian fear conditioning¹¹² and in the DG of the hippocampus in response to corticosterone challenge¹¹³. While the relationship between DNA hypomethylation of these genes in blood and regulation of these genes in the brain is not yet clear, blood-based DNA methylation levels in *ZFP57* and *RNF39* may serve as brain-relevant peripheral biomarkers indicative of trauma-induced susceptibility for PTSD.

In contrast to these male-specific findings, a recent study set out to test the hypothesis that individual variation in response to estrogen levels contributes to fear regulation and PTSD risk in women¹¹⁴; this hypothesis is especially notable given the twofold increased risk of PTSD in women compared to men³. Drawing again on participants from the Grady Trauma Project, and focusing on genes with demonstrated expression in blood, the investigators identified a single CpG site that associated with PTSD following Bonferroni correction, which fell within the histone deacetylase 4 (*HDAC4*) gene: methylation was higher in PTSD cases than controls; and higher methylation at this site was associated with lower estradiol levels. Because *HDAC4* had been linked in previous studies to learning and memory-related processes, the authors further sought to determine the relation between molecular variation at this locus and fear-related traits using both human and animal approaches. Available GWAS data indicated that subjects with the CC genotype at rs7570903, which associated with *HDAC4* methylation and expression levels, showed enhanced fear expression and that it interacted with PTSD diagnosis to predict increased fear conditioning and higher fear-potentiated startle during the early extinction phase; moreover, this risk genotype was associated with increased resting-state functional connectivity in fear-memory-related regions of the brain, specifically the amygdala and cingulate cortex. Follow-up animal

experiments specifically targeting the amygdala suggested that estrogen levels mediate the effects of tone-shock exposure on alterations in *HDAC4* mRNA expression, with higher estrogen apparently buffering against tone-shock related regulation of this gene. Taken together, these results indicate that estrogen levels (at time of tone-shock exposure) influence susceptibility to fear-related phenotypes in a manner that may mediate risk for PTSD in females, and that these PTSD-associated epigenetic effects are also apparent in peripheral blood.

Towards Investigating Neuroepigenetics of PTSD in Humans

While the diagnosis of PTSD is most directly associated with brain function, epigenetic studies of PTSD in humans have been limited to peripheral tissues. Insights on epigenetic alterations in the brain have relied on animal models of PTSD, which are limited in translatability and still require validation in humans. In other stress-related psychiatric conditions, postmortem human brains have been investigated to validate and complement neuroepigenetic insights from animal studies (e.g.¹¹⁵). Although postmortem brain studies may be used to understand the neuroepigenetics of PTSD in the future, these studies cannot be longitudinal in design, and therefore will not be able to inform whether any epigenetic differences exist prior to onset of PTSD (indicative of pre-existing susceptibility factor), develop in response to trauma exposure, or develop during the course of PTSD. A current approach that is used to associate structural differences and functional activity in the brain with peripheral epigenetic profiles (e.g.^{116–119}) can utilize a longitudinal design in living individuals to identify the most robust and brain-relevant biomarkers¹²⁰ and distinguish whether the biomarker is indicative of risk for developing PTSD, response to trauma, pathophysiology, or treatment efficacy. In fact, since epigenetic biomarkers need to come from accessible, peripheral tissue for clinical utility, this approach may be more relevant for biomarker discovery.

An exciting direction for studying the neuroepigenetics of PTSD relies on the development of imaging technologies that may measure and localize activity of epigenetic processes in living humans. Development of radiotracers that penetrate the blood-brain-barrier and bind to key epigenetic enzymes will enable such measurements using PET imaging. In fact, [¹¹C]Martinostat has been characterized and used to quantify the distribution of class I HDAC expression in various tissues including the brain, due to its efficient blood-brain barrier penetrance^{121,122}. This radiotracer was also used in the first effort to acquire *in vivo* measurements of neuroepigenetic regulation in living humans¹²³. However, an important confound to note is that [¹¹C]Martinostat, which selectively binds HDAC isoforms most implicated in regulation of neuroplasticity and cognitive function, can induce epigenetic alterations itself. In neural progenitor cell culture, [¹¹C]Martinostat induced changes in expression of genes associated with synaptic plasticity (i.e., *BDNF* and *synaptophysin*) and neurodegeneration (i.e., progranulin), as well as increased histone acetylation¹²³. Additionally, differential expression of HDAC subtypes have been noted in postmortem brain of subjects with other psychiatric disorders, and were shown to be disorder, HDAC subtype, and brain region specific¹²⁴. However, [¹¹C]Martinostat is unable to distinguish between these HDAC subtypes (i.e., isoforms 1,2,3). Therefore, postmortem human brain studies of dysregulated HDAC isoform expression in PTSD will be needed to benchmark

HDAC isoform-specific density and distribution in different regions of the brain. Further developments stemming from this tracer¹²⁵, as well as synthesis and preclinical testing of other promising epigenetic radiotracers, such as [18F]-TFAHA (selective for HDAC IIa)¹²⁶, have been reported and may improve and expand our capabilities for *in vivo* epigenetic measurements.

PET imaging of neuroepigenetic regulation can also be conducted at the same time as structural and functional MRI imaging using an MR-PET scanner to acquire a variety of other measurements that inform brain structure and function. For example, white tract matter integrity, measured using diffusion tensor imaging (DTI), has been found to correlate with epigenetic aging, calculated from DNA methylation profiles of peripheral blood, and to share common genetic influences with this peripherally-derived measure¹²⁷. This is relevant to PTSD, which is associated with increased risk for a number of age-related comorbidities and hypothesized to accelerate cellular aging¹²⁸.

In fact, lifetime PTSD severity has been found to be associated with accelerated aging^{129,130} based on one DNA methylation-based calculation of cellular age¹³¹, as well as reduced neural integrity (in the genu of the corpus callosum), as indexed by DTI¹²⁹. Since cumulative life stress has been shown to be strongly predictive of accelerated epigenetic aging¹³², the persistent symptoms and residual effects of PTSD may themselves be chronic stressors that promote accelerated epigenetic aging. This is in contrast to current PTSD diagnosis or severity, which has not been found to associate with accelerated epigenetic aging¹³⁰. As a matter of fact, a longitudinal study of military personnel reported an apparent reversal of epigenetic aging during development of PTSD symptoms¹³³, which suggests an initial attempt at compensation before loss of control and accelerated epigenetic aging.

Additionally, life stressors later in life may influence epigenetic aging more strongly than those earlier in life, possibly as a result of cumulative “wear and tear” on the epigenome that render older chronically stressed individuals more susceptible to epigenetic aging than younger individuals¹³². It is also worth noting that earlier stressors (e.g., childhood traumas) may moderate susceptibility to later life stressors in complex ways (i.e., either enhance or suppress susceptibility) such that the cumulative effects that contribute to epigenetic remodeling over a lifespan are not linear and depend on the stage/context of each contributing life stressor¹³⁴. Future longitudinal studies that integrate neuroimaging markers with epigenome-based metrics (e.g., epigenetic aging measures from immune cells) can help advance our understanding of how life stressors build on each other to shape an individual’s immunological age and neural integrity over time. This approach would be well-suited for following at-risk children who are chronically exposed to violence¹³⁵ and assessing whether interventions (e.g., supportive family environments¹³⁶) reverse accelerated epigenetic aging and buffer against detrimental effects of life stressors.

In summary, collection of multidimensional neuroimaging data with epigenomic profiling of peripheral tissue holds particular promise for characterizing the neuroepigenetic basis of PTSD from a life course perspective. Specifically, this relatively non-invasive approach can be used at multiple timepoints (preferably using a prospective design to collect before and after trauma exposure) to discover the trajectory of PTSD-associated epigenetic changes in

different brain regions and determine how they deviate from trauma-exposed non-PTSD and non-trauma exposed controls. Moreover, comprehensive history and phenotype information including lifetime exposures to stressors and traumas (especially during childhood), type of trauma, environmental conditions (e.g., socioeconomic status), social stressors and support, and comorbid conditions, are critical to include in the multidimensional dataset. Given the heterogeneous presentation of PTSD across different symptom clusters, this phenotype information will be essential for differential diagnosis and identification of subtypes of PTSD based on underlying pathophysiology. The RDoC initiative provides a framework by which this multidimensional data can be leveraged to understand individual posttraumatic conditions based on these neurobiological and neuroepigenetic metrics and symptoms^{137,138}. Through these continuing advances, future research should be able to better unpack the neuroepigenetic basis of PTSD in living individuals, thereby improving prospects for treatment and prevention.

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Figure 1.

Schematic diagram of neural circuitry involved in fear learning. The hippocampus, which is involved in contextual processing, has projections to the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC), namely the prelimbic (PL) and infralimbic (IL) cortices. These projections are necessary for contextual fear memory retrieval and for modulating renewal and suppression of fear expression after extinction via PL and IL projections, respectively. The IL region of the mPFC is important for fear extinction learning and sends projections to the intercalated neurons (ITC), directly and indirectly, which inhibit excitation of the central amygdala (CeA). The PL region projects to the BLA to signal excitation of the CeA. Outputs from the CeA drive expression of the fear response. Refer to reviews for more detail^{21,25,26}.

Figure 2.

(A) Schematic representation of general experimental paradigm used to determine the effect of stress on fear learning and memory. Stress exposure prior to fear conditioning enhances conditional fear responding and impairs fear extinction learning (i.e., extinction acquisition and/or extinction retrieval), which corresponds to increased fear behavior. (B) Tables summarizing discussed epigenetic changes at time points noted in (A), by brain region (i.e., hippocampus [blue], amygdala [orange], and prefrontal cortex [green]). Timing of manipulation is indicated by syringe (orange⁸²; blue⁸⁰).

Figure 3.

Summary of 43 human-based epigenetic studies of PTSD published as of July 2017, enumerated and categorized according to relevant biological system or Epigenome-wide association (EWAS) approach: Hypothalamic-Pituitary Adrenal (HPA) Axis^{105,108,116,119,139–150}; EWAS^{91–96,151–155}; Age-related CpGs^{129,132,133}; Serotonergic system^{156,157}; Neurotrophin^{117,158}; Dopaminergic System¹⁵⁹; Catecholaminergic system¹⁰²; DNA methylation machinery¹⁶⁰; Estrogen-responsive genes¹¹⁴; Immune Function¹⁶¹; Other^{162–165}.