

## Special Issue: Neuropsychiatric Disorders

# Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder

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**Posttraumatic stress disorder (PTSD) is an anxiety disorder that can develop after a traumatic experience such as domestic violence, natural disasters or combat-related trauma. The cost of such disorders on society and the individual can be tremendous. In this article, we review how the neural circuitry implicated in PTSD in humans is related to the neural circuitry of fear. We then discuss how fear conditioning is a suitable model for studying the molecular mechanisms of the fear components that underlie PTSD, and the biology of fear conditioning with a particular focus on the brain-derived neurotrophic factor (BDNF)-tyrosine kinase B (TrkB), GABAergic and glutamatergic ligand-receptor systems. We then summarize how such approaches might help to inform our understanding of PTSD and other stress-related disorders and provide insight to new pharmacological avenues of treatment of PTSD.**

## Introduction

Irrational fear is a major impediment to success and productivity. In 1933, when Franklin D. Roosevelt acknowledged 'the only thing we have to fear is fear itself', he was commenting on the economic future of the USA, but unreasonable over-generalized fear can have dramatic effects on all aspects of one's life. Over-generalized fear is one of the biggest symptoms of anxiety disorders, in particular disorders of fear regulation, including phobia, panic disorder and posttraumatic stress disorder (PTSD). PTSD is an example of how excessive fear can impair quality of life. Although fear learning is an evolutionarily advantageous response mechanism, when fear becomes too generalized, this mechanism might not only be unproductive but harmful. PTSD is a disorder where learned fear due to a traumatic event becomes generalized to situations that would normally be considered safe and results in autonomic hyperarousal in inappropriate situations.

Three types of symptoms are prevalent in PTSD: reexperiencing, avoidance and hyperarousal. Reexperiencing symptoms involve flashbacks, nightmares and frightening thoughts about the trauma, which can result in physical symptoms including headaches, pains and other symptoms

of somatization. Avoidance symptoms include avoiding reminders of the experience, feeling emotionally numb, losing interest in previously enjoyable activities and deficits in learning and memory. These symptoms might cause a person to change his or her personal routine. Finally, hyperarousal symptoms include being easily startled, feeling tense, having difficulty sleeping and/or having angry outbursts. Reminders of the traumatic event usually trigger reexperiencing and avoidance symptoms whereas hyperarousal symptoms might be present more continuously [1–6].

There is variability in the prevalence and severity of PTSD [3]. Trauma is necessary but not sufficient for the precipitation of PTSD. In fact one of the most critical current questions is why some trauma victims develop PTSD (between 5 and 30%) [1,3,4] whereas others experiencing the same trauma appear to be resilient. In addition, those who meet the criteria for PTSD vary widely in their symptom severity and in the type of symptoms they experience [1,3–8]. A variety of factors contribute to the magnitude of PTSD symptoms, including an individual's genetic makeup, predisposition, social support network and early life experiences [9–12] (Box 1). In other words, these factors might determine an individual's resilience to trauma. Studying what accounts for this resilience in certain individuals could help target treatments and the prevention of PTSD in trauma victims predisposed to develop PTSD. Understanding the neurobiological mechanisms of PTSD as well as developing more rapid and cost-effective treatments is of vital importance. The

avoidance = avoiding reminders of the experience  
feeling emotionally numb, losing interest in previously enjoyable activities, deficits in learning & memory

hyperarousal = being easily startled, feeling tense, having difficulty sleeping, having angry outbursts

## Glossary

**Classical conditioning:** a learning paradigm that pairs a neutral/conditioned stimulus (CS) with an unconditioned stimulus (US) that evokes a reflex or unconditioned response (UR) until the neutral stimulus evokes the same conditioned response (CR) in the absence of the US.

**Contextual conditioning:** a model of fear conditioning based solely on the context and not a discrete cue such as a light or a tone.

**Extinction:** the conditioning phenomenon in which a previously learned response to a cue is reduced when the cue is presented in the absence of a previously paired aversive or appetitive stimulus.

**Pavlovian fear conditioning:** a version of classical conditioning where the CS (e.g. tone, light, odor) is paired with an aversive US (e.g. foot shock, air blast) that evokes a CR (e.g. freezing, acoustic startle response or autonomic arousal).

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PTSD --> fear generalized to situations that would normally be considered as safe --> automatic hyperarousal in inappropriate situations

PTSD --> reexperiencing, avoidance, hyperarousal

Reexperiencing = flashbacks, nightmares, frightening thoughts about the trauma

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### Box 1. Genetic association studies in PTSD

**How it works:** these studies compare the DNA of two groups of participants: trauma victims with PTSD and trauma victims without PTSD. Each person gives a sample of cells from their cheek, saliva or blood. DNA is extracted from these cells and gene chip analyses are performed. Rather than reading DNA sequences, these systems use SNPs that are markers for regional DNA variation. If genetic variations are more frequent in the affected participants, then the variations are said to be associated with the disorder.

BDNF = brain-derived neurotrophic factor (= representative protein related to neuronal differentiation, maturation, & survival)

#### Some replicated genetic associations found in PTSD

##### BDNF (Val66Met) SNP

Function: neurotrophic factor  
Result of polymorphism:

- Met allele has been shown to have altered trafficking and secretion in neurons compared to Val allele [51].
- Met/Met carriers showed increased medial temporal lobe activation (perhaps compensatory) during episodic and encoding/retrieval tasks [52].
- Greater recruitment of amygdala and PFC activity in Met/Met carriers during memory formation and retrieval of biologically relevant stimuli [53].
- Met/Met carriers exhibited impaired extinction learning, which was correlated with altered activation of the amygdala, PFC and the hippocampus [54].

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BDNF Val66Met SNP = valine-to-methionine substitution at codon 66 of the BDNF gene single nucleotide polymorphism

<https://pubmed.ncbi.nlm.nih.gov/32844059/>

##### Serotonin transporter (SERT): short versus long allele

Function: serotonin transport/reuptake

Result of polymorphism:

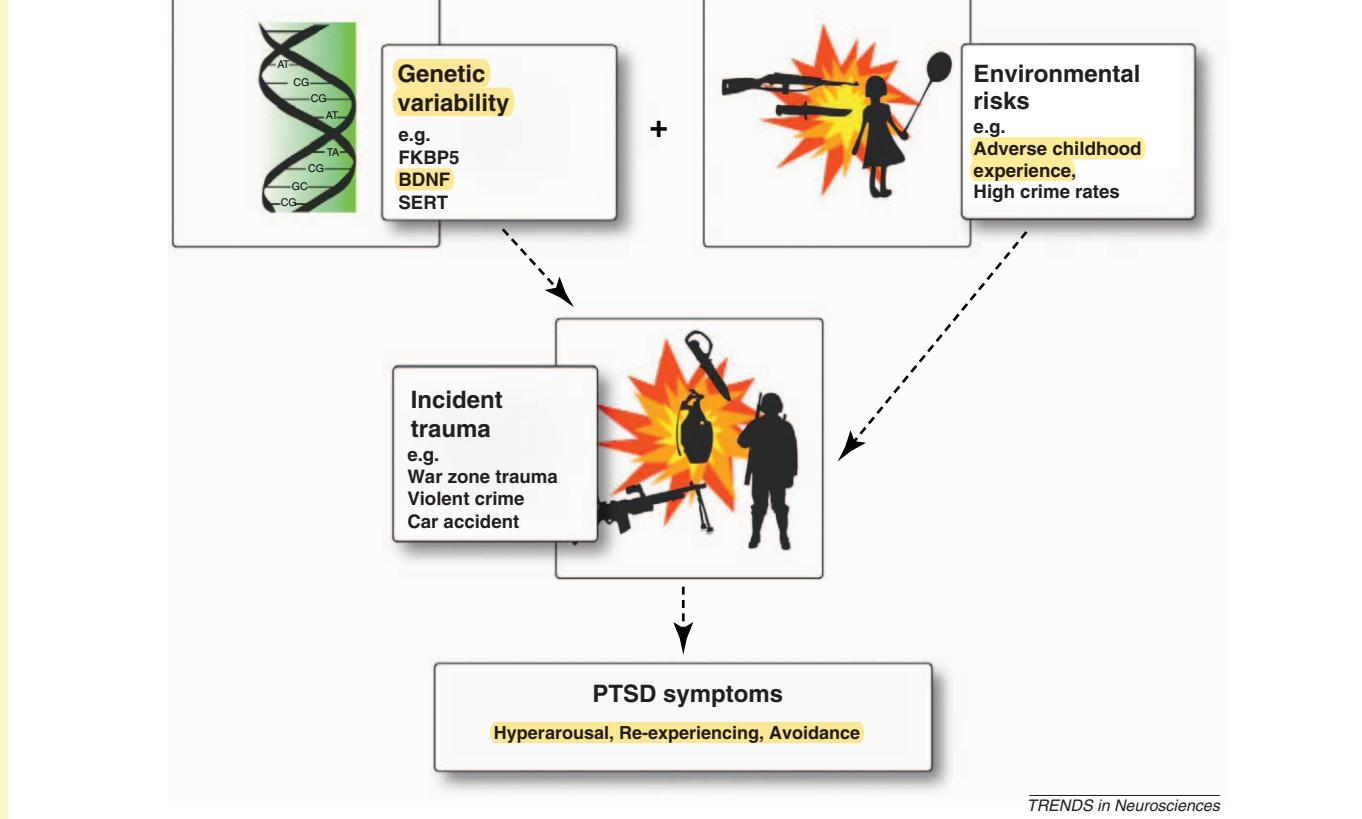
- Different alleles have been associated with altered SERT gene expression/translation [158–160].
- Findings have been reported in individuals for an increased risk of PTSD with both the long [158,159] and short allele [158,160].
- Recent data suggest that the short allele is associated with decreased risk of PTSD in low-risk environments (e.g. low crime/unemployment rates) but increased risk of PTSD in high-risk environments [158]. This suggests that environment modifies the effect of serotonin transporter-linked polymorphic region (5-HTTLPR) genotype on PTSD risk (Figure 1).

##### FK506-binding protein 5 (FKBP5)

Function: glucocorticoid chaperone protein

Result of polymorphism:

- PTSD associated with differential FKBP5 mRNA and protein expression [161].
- No main effect of FKBP5 genotype on PTSD [9].
- FKBP5 SNPs interact with child maltreatment history as a predictor of the severity of adult PTSD symptoms [9].
- FKBP5 SNPs might contribute to increased sensitivity of the amygdala/HPA axis response to adult stress.
- The serine protease neuregulin is critical for stress-related plasticity in the amygdala by regulating EphB2-NMDA-receptor activation of FKBP5 expression [162].



**Figure 1.** Genetic and environmental factors influence the risk for developing PTSD in certain individuals as well as the severity of PTSD symptoms.

current review addresses recent molecular approaches to understanding PTSD using animal models of fear, limitations of these models and speculation about how these models might lead to better treatment and understanding of PTSD and other fear-related disorders.

#### Pavlovian fear conditioning as a model for understanding the underlying mechanisms of pathological fear responses

The neural structures important to PTSD belong to the limbic system, a region important for emotional processing

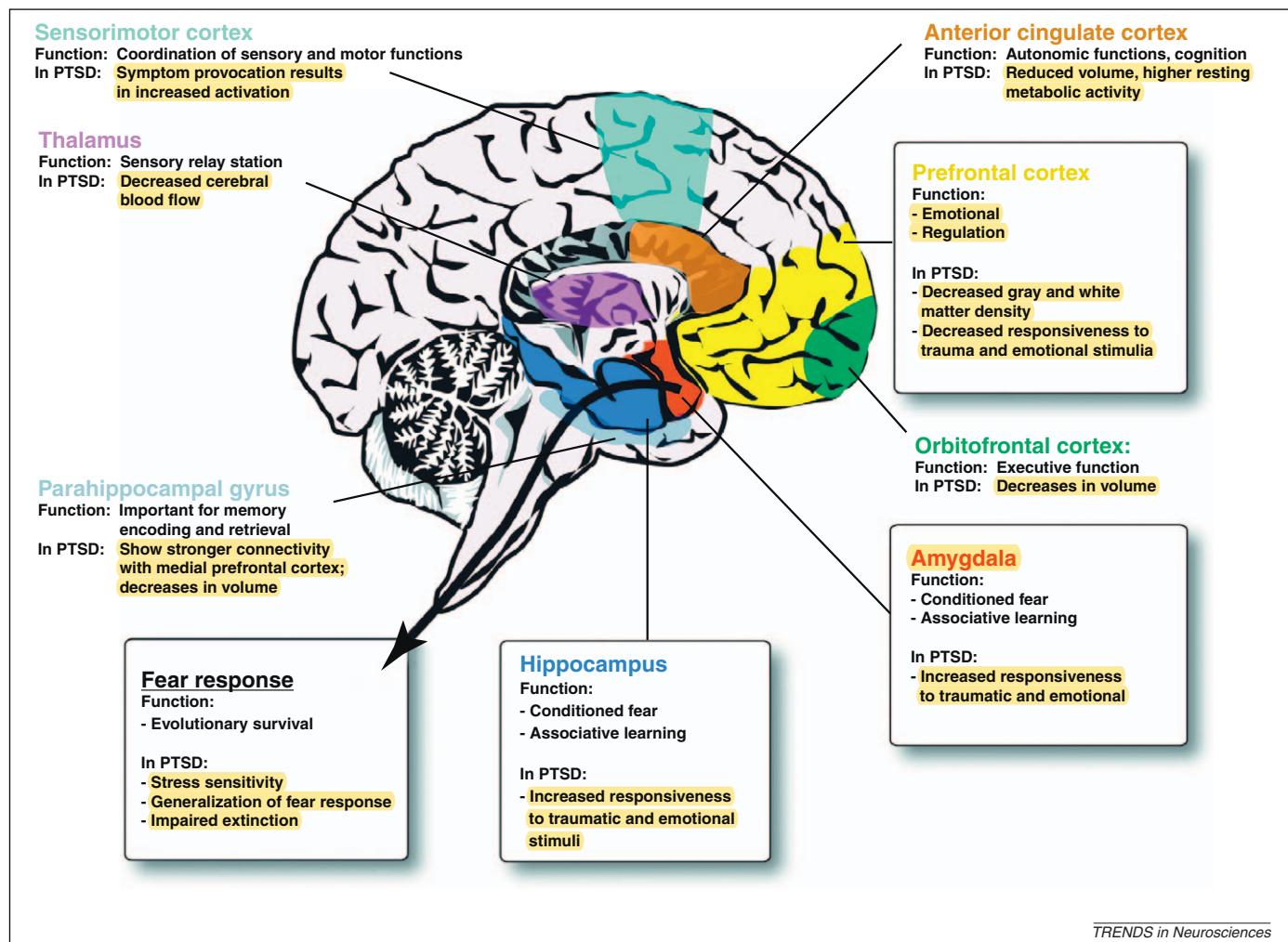
in both humans and animals [13]. The three regions within the limbic system most clearly altered in PTSD include the amygdala, the hippocampus and the prefrontal cortex (PFC). The amygdala regulates learned fear in animal and human studies of Pavlovian fear conditioning (see [Glossary](#)) and receives projections from the hippocampus and PFC [14–18]. Subjects with PTSD show reduced activation of the PFC and hippocampus, which might coincide with reduced top-down control of the amygdala, possibly resulting in a hyper-responsive amygdala signal to fearful stimuli [14]. This might result in the disordered fear regulation in PTSD and other fear-related disorders. Other regions involved with PTSD include the parahippocampal gyrus, orbitofrontal cortex, the sensorimotor cortex, the thalamus [7] and the anterior cingulate cortex (Figure 1) [19–21].

Patients with PTSD show markedly different responses to fear conditioning paradigms relative to trauma victims without PTSD [22–31]. They demonstrate behavioral sensitization to stress [22–24] and over-generalization of the

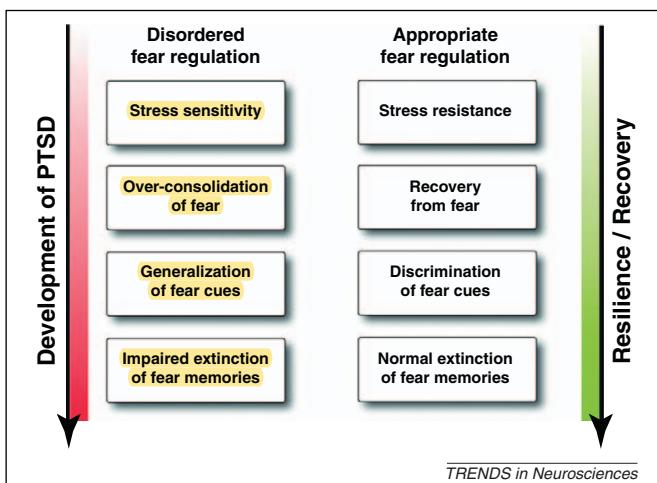
conditioned stimulus (CS)–unconditioned stimulus (US) response [25,26]. Such patients show impaired extinction of CS–US pairings [27–29] and show impaired fear inhibitory learning [31]. It is thought that this altered fear response might result in the intrusive memories and flashbacks, enhanced avoidance of reminder cues and autonomic hyperarousal seen in PTSD [31,32]. The neural circuitry of fear conditioning is conserved across most vertebrate species and its behavioral readout is both quick and robust [33,34]. Therefore, fear conditioning is a tractable method of studying the fear response underlying PTSD. Many of the molecular tools that have been developed to study behavior in rodents can be applied to study mechanisms of fear dysregulation and, therefore, to develop new therapeutics that might prove valuable for the treatment of PTSD.

Evidence from animal models and human neuroimaging studies suggest that one of the underlying mechanisms of PTSD might be aberrant synaptic plasticity [7,15,35–44]. Synaptic plasticity describes the changes that occur at the

PTSD = behavioral sensitization to stress, over-generalization of CS-US response, impaired extinction of CS-US pairings, impaired fear inhibitory learning



**Figure 1.** A schematic of the human brain illustrating how the **limbic system** is involved in posttraumatic stress disorder (PTSD). The **prefrontal cortex (PFC)** and the **hippocampus** both have dense connections to the **amygdala**, which is important for **conditioned fear** and **associative emotional learning**. The PFC is thought to be responsible for **reactivating past emotional associations** and is decreased in both responsiveness and density [7,8,14,15]. The hippocampus is thought to play a role in explicit memories of traumatic events and in **mediating learned responses to contextual cues**; in PTSD, the hippocampus is decreased in volume [150] and responsiveness to traumatic stimuli [20,150]. The top down control of the amygdala by the hippocampus and PFC might result in the increased activation of the amygdala, as is observed in subjects with PTSD [7,8,14,15]. The **end result of these neuroanatomical alterations is increased stress sensitivity, generalized fear responses and impaired extinction**. Other regions including the anterior cingulate cortex, the orbitofrontal cortex, the parahippocampal gyrus, the thalamus and the sensorimotor cortex also play a secondary role in the regulation of fear and PTSD [151].



**Figure 2.** Disordered fear regulation in posttraumatic stress disorder (PTSD). Individuals with PTSD typically show increased sensitization to stress, over-generalization of fear to irrelevant stimuli and impaired extinction of fear memories. Individuals who demonstrate resilience to PTSD, and/or who recover from traumatic/stressful experiences, are able to discriminate between fearful and non-fearful stimuli, as well as displaying normal extinction of fear memories.

synapse with prolonged synaptic activity. Such changes are physiological, morphological and molecular in nature. Synaptic plasticity is hypothesized to be the underlying basis of learning and memory [35–45]. Behaviorally, subjects with PTSD show increased sensitization to stress, over-generalization of fear associations and failure to extinguish learned fear (Figure 2) [22–31]. Animal models that mimic these behavioral abnormalities, such as animals trained in the fear conditioning or extinction learning paradigms, require synaptic plasticity [35–44]. Therefore, impairment of fear or extinction processes in PTSD might be indicative of impaired synaptic plasticity. Much is known about the molecular mechanisms of synaptic plasticity, and understanding how PTSD might be a disorder of synaptic plasticity within emotional circuits will provide new avenues for translational research.

There are two practical clinical benefits to understanding the biological mechanisms of PTSD: prevention and treatment. A better understanding of the genetics and underlying molecular mechanisms of PTSD will hopefully lead to better predictions about which individuals might be more susceptible to developing PTSD after trauma through genetic, biomarker and psychological screening. In addition, knowledge of the molecular underpinnings of PTSD will point towards novel molecular targets for drug development. By generating drugs that activate these molecular mediators of plasticity, one might be able to enhance extinction of inappropriate fear associations or even prevent development of fear associations in at-risk individuals. This area of research shows great promise for potential new approaches to treat PTSD symptoms.

PTSD =  
fear  
conditioning  
= impaired  
synaptic  
plasticity  
within  
emotional  
circuits

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Under-  
standing  
molecular  
mechanisms  
of PTSD  
--> drug  
development  
(-->  
enhance  
extinction  
of inappro-  
priate fear  
associations  
or even  
prevent  
development  
of fear  
associations)

**Neurotrophic mechanisms of synaptic plasticity in fear conditioning**  
The brain-derived neurotrophic factor (BDNF)-tyrosine kinase B (TrkB) pathway provides one example of a ligand-receptor system that underlies synaptic plasticity and has also been implicated in both PTSD in humans and in animal models of fear conditioning, extinction and

inhibitory learning. Peripheral plasma and serum studies [46–48] as well as genetic studies have directly linked BDNF to PTSD [49]. In addition, transgenic, molecular and behavioral studies in rodents have provided insights into the underlying mechanisms of BDNF signaling in PTSD. There is burgeoning evidence for an association between a single nucleotide polymorphism (SNP) in the BDNF gene (Val66Met) and various psychiatric disorders, including depression and schizophrenia [49,50]. This mutation is thought to alter BDNF stability and activity-dependent secretion, hence leading to dysfunctional BDNF signaling [51]. Although there is limited evidence for a role of the Val66Met polymorphism in PTSD, the Val66Met polymorphism might also result in altered memory function [50–55]. BDNF (Met/Met) carriers showed increased medial temporal lobe activation during episodic and encoding retrieval tasks [52]. Another study described greater recruitment of amygdala and PFC activity in Met/Met carriers during memory formation and retrieval of biologically relevant stimuli [53]. Finally, BDNF (Met/Met) carriers exhibited impaired extinction learning, which was correlated with altered activation of the amygdala, PFC and the hippocampus [54–56]. Together these data suggest that this polymorphism might play a role in activation of the limbic system during memory formation and emotionally-relevant learning.

Humanized BDNF (Val66Met) knock-in mice with the Met/Met phenotype show increased anxiety-related behaviors compared to Val carrier mice when placed in stressful settings [57,58]. BDNF (Met/Met) mice and humans carrying the Met allele show impaired extinction learning after fear conditioning [56,59]. Together these studies suggest that the transgenic mice share a similar phenotype to individuals at risk for PTSD in that they appear to be more sensitive to stress/anxiety and have impaired extinction of conditioned fear. In addition, BDNF (Met/Met) mice showed impaired NMDA receptor-dependent synaptic plasticity in the hippocampus [60]. It has not been reported whether these mice show impaired plasticity in the amygdala and PFC, although the extant data support the idea that PTSD is a disorder of aberrant plasticity mechanisms and that these mechanisms are regulated by BDNF signaling.

BDNF-TrkB signaling has been shown to be necessary for various aspects of fear conditioning and extinction in all three of the regions implicated in PTSD: the amygdala, the hippocampus and the PFC [61–73]. In the amygdala, BDNF transcription is increased during the consolidation period 2 hours after fear conditioning [60–63]. Inhibiting BDNF signaling in the amygdala impairs both the acquisition and consolidation of fear conditioning [67] and the consolidation of extinction [66]. In addition, an increase in BDNF was observed after the normal window of consolidation at around 12 hours after fear conditioning and this peak in BDNF expression was shown to be crucial for persistence of the fear memory [68]. Recent evidence suggests that one effect of BDNF activation of TrkB is to lower the threshold for synaptic plasticity to occur. In single cell slice physiology studies, the threshold for LTP induction in BLA principal neurons is critically dependent on the level of dopamine in the extracellular milieu and the synergistic

mutation =  
single  
nucleotide  
polymorphism  
(SNP) in the  
BDNF gene  
(Val66Met)

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polymorphism = one or  
two variants of a  
particular DNA sequence  
(<https://www.genome.gov/genetics-glossary/Polymorphism>)

BDNF carriers  
= impaired  
extinction  
learning (=  
even as the  
US, the real  
threat, is not  
present, there  
is no extinction  
of fear triggered  
by a CS)

"Transgenic mice  
are mouse  
models that  
have been  
altered by the  
stable insertion  
of DNA called  
transgenes."

(<https://www.genetargeting.com/transgenic-mouse-models.com/>)

aberrant =  
abnormal  
plasticity =  
returning to  
normal (ability  
to change)

LTP = Long-term  
potentiation (= a  
persistent increase  
in synaptic  
strength following  
high-frequency  
stimulation of a  
chemical synapse)

BLA =  
basolateral  
nucleus of  
the amygdala

activation of postsynaptic D1 and TrkB receptors [74]. This is consistent with other new data examining thalamo-amygdala LTP processes, which suggest a postsynaptic site of action of BDNF in mediating LTP selectively in the thalamic fear conditioning pathway [75]. Thus, BDNF signaling in the amygdala appears to play a significant role in synaptic plasticity events underlying the consolidation and the persistence of fear memories.

Mice heterozygous for the BDNF deletion (*BDNF*+/−) showed impaired contextual fear conditioning, which could be partially rescued with expression of BDNF in the hippocampus [69]. Mice in which *BDNF* was selectively deleted from the hippocampus did not show impaired acquisition of fear conditioning; however, there was a marked decrease in extinction of conditioned fear [62]. This result suggests that normal hippocampal plasticity is required for normal context-dependent extinction of conditioned fear. Taken together with the findings of smaller hippocampal volumes in subjects with PTSD [62,69], these convergent data suggest that impaired hippocampal function in PTSD might be causally related to these subjects' impairment in extinction of fear memories.

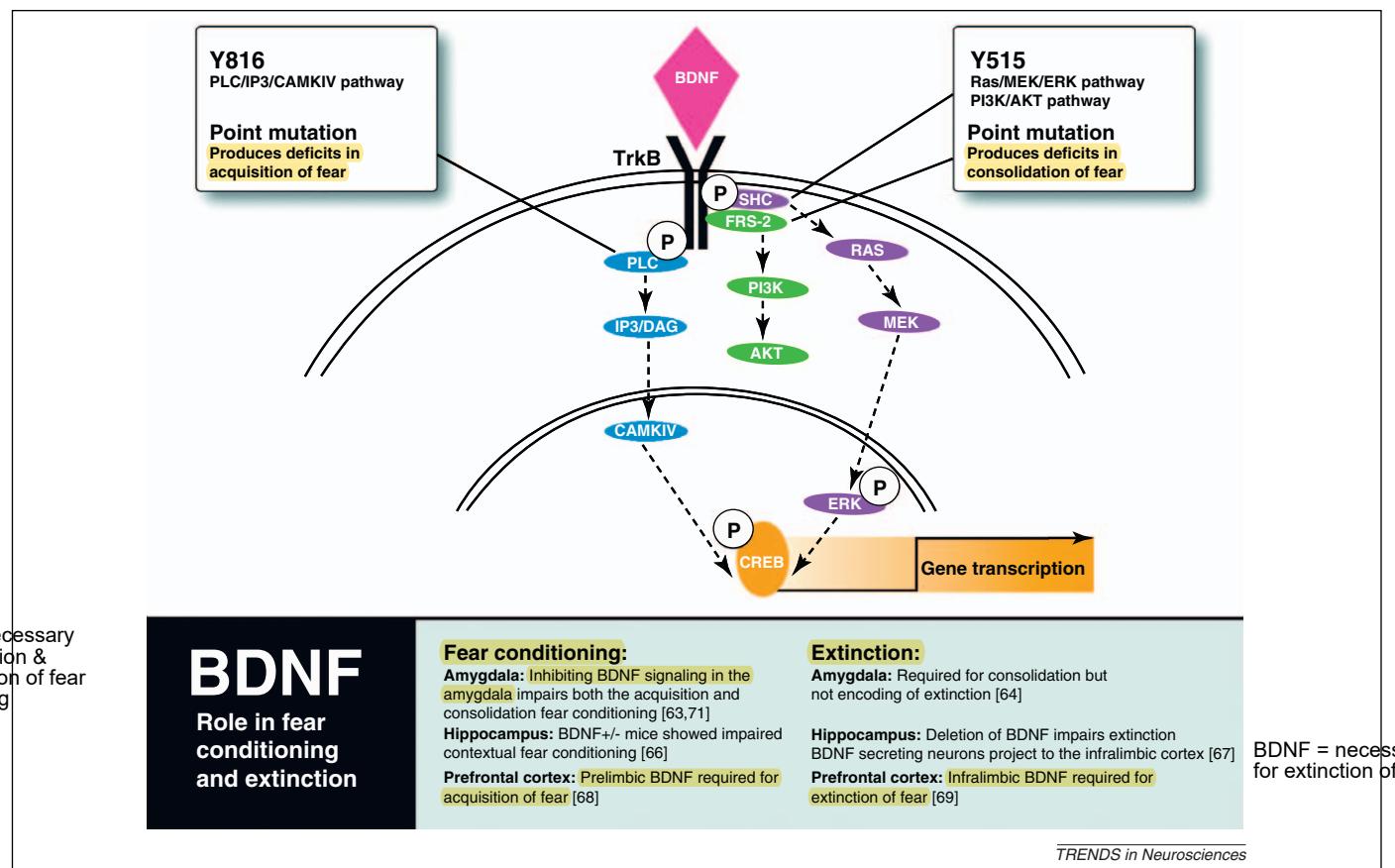
BDNF has also been implicated in differential roles in distinct subregions of the PFC in the retention and in the extinction of learned fear. Genetic deletion of *BDNF*

selectively in the prelimbic area (PL) of the PFC causes impairment in consolidation of learned fear but not extinction [70]. By contrast, infusing BDNF into the infralimbic area (IL) of the PFC resulted in reduced fear expression for up to 48 hours after fear conditioning even in the absence of extinction training but did not erase the original fear memory [71]. Rats with impaired extinction showed less BDNF expression in the IL PFC compared to control rats, and infusing BDNF into the IL prevented extinction failure. These data suggest that BDNF might be a crucial mediator of neural plasticity in both regions. Owing to the differential connectivity and functioning of IL and PL, BDNF in these areas also results in opposite effects. BDNF in the PL is necessary for fear memory formation and expression, whereas BDNF in the IL is apparently necessary for the inhibition, or extinction, of that fear. Thus, BDNF signaling in the PFC plays a critical role in the regulation of fear and emotion and might serve as a target for enhancing extinction in subjects with PTSD.

The TrkB receptor is composed of an extracellular domain that binds BDNF and an intracellular domain that activates signaling pathways through phosphorylation of two tyrosine residues, Y515 or Y816, which activate divergent signaling pathways (Figure 3). Phosphorylation of the Y515 residue allows recruitment of Src homology 2 domain

impaired extinction in rats = less BDNF expression in infralimbic prefrontal cortex compared to control rats

PTSD sufferers  
--> impaired hippocampal function (smaller hippocampal volumes) might cause impairment in extinction of fear memories



**Figure 3.** The brain-derived neurotrophic factor (BDNF)-tyrosine kinase B (TrkB) induced signaling pathway. BDNF binds to the TrkB receptor, resulting in the phosphorylation of two tyrosine sites (Y515 and Y816) on the intracellular domain of the TrkB receptor. Phosphorylation of the Y515 residue allows recruitment of Src homology 2 domain containing/fibroblast growth factor receptor substrate 2 (Shc/FRS-2), which subsequently activates the Ras/mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. By contrast, phosphorylation of the Y816 residue allows recruitment of phospholipase C (PLC), which activates the  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase (CAMK)/cAMP responsive element binding protein (CREB) signaling pathway. Point mutations of the Y515 residue produce deficits in consolidation but not acquisition of fear conditioning [72]. By contrast, point mutations of the Y816 residue produce deficits in acquisition [72]. Evidence exists for a role of BDNF signaling in the amygdala [63,64,71], hippocampus [66,67] and PFC [68,69] with respect to both the consolidation and extinction of fear conditioning.

containing/fibroblast growth factor receptor substrate 2 (Shc/FRS-2) activating the RAS/mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways. By contrast, phosphorylation of the Y816 residue allows recruitment of phospholipase C (PLC), which activates the  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase (CAMK)/cAMP responsive element binding protein (CREB) signaling pathway [76]. Genetic mouse models carrying single point mutations at each of these two sites (Y515F or Y816F) have been developed [72]. *TrkB* (Y515F) knock-in heterozygous mice exhibited deficits in consolidation but not acquisition of fear conditioning, whereas *TrkB* (Y816F) mice exhibited deficits in acquisition [72]. How acquisition and consolidation lead to differential activation of the *TrkB* receptor at the Y515 site versus the Y816 site is currently unclear. Furthermore, it will be of interest to study the different roles of these phosphorylation sites in the extinction of learned fear.

Despite significant evidence suggesting a role for the BDNF-TrkB system in fear-related and other affective disorders, a lack of ligands for the high affinity TrkB receptor has limited progress towards BDNF-related treatments for psychiatric and neurological disorders. However, 7,8-dihydroxyflavone (7,8-DHF) has recently been identified as a relatively specific TrkB agonist that crosses the blood-brain barrier after oral or i.p. systemic administration in mice [61]. It was subsequently demonstrated that amygdala TrkB receptors are activated by systemic 7,8-DHF (5 mg/kg, i.p.) [73]. In addition, systemic 7,8-DHF rescued the fear consolidation deficit observed in prelimbic BDNF knockout mice [70] and enhanced both the acquisition of fear and its extinction in wild type mice [73]. Furthermore, this agonist appears to rescue an extinction deficit in mice with a history of immobilization stress, which might serve as a face-valid animal model of PTSD [73]. These data suggest that 7,8-DHF and other potential TrkB activating ligands might not only be valuable as pharmacological tools for achieving a better understanding of the role of BDNF-TrkB signaling pathways in learning and memory, but also as potential therapeutics for reversing learning and extinction deficits associated with psychopathology.

An additional molecule that has been implicated in synaptic plasticity and BDNF regulation is pituitary adenylate cyclase-activating polypeptide (PACAP). PACAP is known to broadly regulate the cellular stress response, however, it was only recently demonstrated to also have a role in human psychological stress responses, such as PTSD. Specifically, a sex-specific (female) association of PACAP blood levels with fear physiology, PTSD diagnosis and symptoms was observed in a population of heavily traumatized subjects [77]. In addition, a single SNP in a putative estrogen response element within the PACAP receptor (PAC1) was associated with PTSD symptoms in females only. This SNP also associated with enhanced levels of fear discrimination and with levels of PAC1 mRNA expression in human cortex. Methylation of the *PAC1* gene in peripheral blood was also found to be significantly associated with PTSD [77]. Note that an increasing body of literature is suggesting an important role for epigenetic regulation (DNA methylation and histone

modification) of amygdala-dependent fear processes in animal models (e.g. [78]). Complementing these human findings, PAC1 mRNA expression was induced with either fear conditioning or estrogen replacement in rodent models [77]. These data suggest that perturbations in the PACAP-PAC1 pathway are involved in abnormal stress responses underlying PTSD, and that some of the sex-specific differences in PTSD risk/resilience [79] might be in part due to estrogen modulation of this pathway.

Fear -->  
reduces  
GABAergic  
signaling

### GABAergic inhibitory regulation of neuronal circuits in fear conditioning

GABAergic inhibitory control is crucial for the precise regulation of consolidation, expression and extinction of fear conditioning [80–82]. Fear conditioning results in a reduction in GABAergic signaling in the basolateral nucleus of the amygdala (BLA) relative to non-fear conditioned controls [83] and genetic deletion of the  $\alpha 1$  subunit of the GABA<sub>A</sub> receptor enhances auditory fear learning [84]. Many of the early papers used GABA agonists as a method of inactivating specific brain regions to determine their role in behavior. GABAergic inactivation of the amygdala, hippocampus, PFC and regions of the striatum resulted in impairments in various aspects of conditioned fear [85–87]. In addition, GABAergic inactivation of the infralimbic cortex, BLA or ventral hippocampus also impaired fear extinction [86,88,89]. However, GABAergic signaling is more than a methodological tool for inactivating regions of the brain but appears to maintain tight regulatory control over microcircuits in a region and cell-type specific manner.

GABAergic = any chemical that modifies the effects of GABA in the body or brain

GABA = gamma-aminobutyric acid (= the primary inhibitory neurotransmitter that slows down or weakens signals in the brain); has a role in mood management; can be found in food (such as broccoli, cabbage, cauliflower, brussels sprouts, soy beans, mushrooms, spinach, tomatoes, peas, chestnuts, sweet potatoes, rice/brown rice)

<https://www.livestrong.com/article/478780-a-list-of-foods-with-the-highest-gaba/>

Two recent papers have outlined how GABAergic inhibitory microcircuits might regulate acquisition and expression of fear memories in the central nucleus of the amygdala (CEA). It was originally thought that associative learning primarily occurs in the BLA, whereas the CEA mainly controlled the expression of fear [90]. Such regulation of fear expression occurs via projections from central amygdala output neurons, which are mainly located in the medial subdivision (CEm), to the brainstem and hypothalamus [90]. However, a role for the CEA in fear acquisition has been demonstrated [90]. Activation of the CEm in mice by pharmacological and physiological techniques was found to result in strong and reversible freezing responses [90]. Inactivating the lateral division of the CEA (CEL), but not the CEm, was found to induce unconditioned freezing as well as impairing fear conditioning. From these results, it was concluded that neuronal activity in the CEm is necessary and sufficient for driving the freezing response but that the CEL is required for the acquisition of fear and produces tonic inhibitory control of the CEm, which is reduced during presentation of the conditioned stimulus (CS+) [90].

Moreover, the above study also identified two distinct subpopulations of inhibitory GABAergic neurons in the CEL [90]. These neuronal subpopulations were termed CEL 'on' and 'off' neurons based on their response to fear conditioning. CEL 'on' neurons acquired an excitatory response to the CS+ during and after fear acquisition, whereas CEL 'off' neurons showed decreased responses to the CS+ during and after fear acquisition. CS evoked excitation of CEL 'on' neurons began before the CEL 'off' neurons, and

both 'on' and 'off' neurons sent inhibitory projections to the CEm [90]. CS evoked inhibition of 'off' neurons started immediately prior to excitation of CEm neurons, indicating that increases in CEm firing might be due to a reduction of inhibition from CEL 'off' neurons. It is also probable based on the short onset latency of the CS-evoked excitation of CEL 'on' neurons that they receive direct input from the sensory thalamus. The CEm also receives thalamic input [90], which might be inhibited by feed forward inhibition through the CE 'on' pathway. Based on this physiological data, it is hypothesized that fear conditioning leads to a shift in the balance of activity between distinct classes of CEL neurons, which ultimately regulates the activity of CEm firing [90].

A second recent study has added to the understanding of CEA inhibitory microcircuits by molecularly defining two subtypes of inhibitory neurons in the CEL by the presence or absence of the  $\delta$  isoform of protein kinase C (PKC-  $\delta$ ) [91]. Using molecular and genetic approaches, this study was able to map the functional connectivity of PKC-  $\delta$  + and PKC-  $\delta$  - neurons. Specifically, optogenetic targeting was employed to examine the effect of reversibly silencing PKC-  $\delta$  + neurons on the activity of CEL 'on', CEL 'off' and CEm neurons. PKC-  $\delta$  + neurons were found to be predominantly late firing neurons, which reciprocally inhibit PKC-  $\delta$  - neurons. Inactivation of PKC-  $\delta$  + neurons evoked action potentials in the CEm output neurons. In addition, tonic activity of CEL 'off' units was strongly suppressed by the inactivation of PKC-  $\delta$  + neurons. Taken together, these findings suggest that the PKC-  $\delta$  + neurons are likely to be the CEL 'off' neurons [91] (Figure 4).

Another recent study observed that temporally precise optogenetic stimulation of BLA terminals in the CEA exerted an acute, reversible anxiolytic effect [92]. These

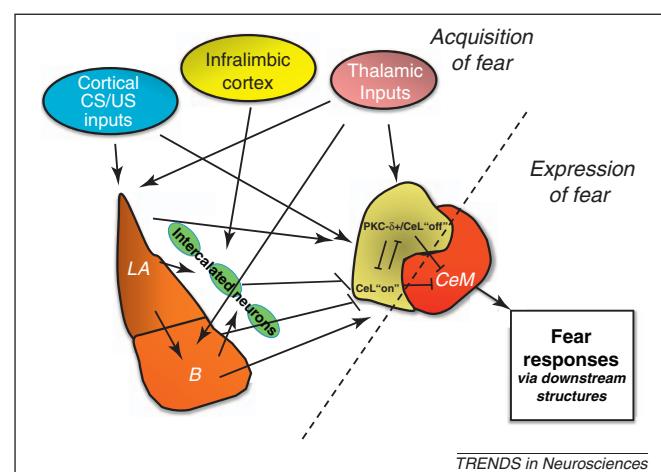
results implicate specific BLA-CEA projections as critical circuit elements for acute anxiety control in the mammalian brain.

Together, these recent papers provide new insight into the role of GABAergic inhibitory microcircuits in the acquisition and expression of fear conditioning. One outstanding question from this research is: if both CEL 'off' and CEL 'on' units send inhibitory projections to the CEm, why is CEm activity increased rather than decreased after fear conditioning? This might be due simply to a balance between on and off neuron firing, i.e. the effect of decreased CEL 'off' firing is greater than the effect of increased CEL 'on' firing. Another reason could be that the CEL 'on' neurons project to a different subpopulation of CEm neurons. Such recent findings add another level of control to the acquisition of fear. Not only is the BLA complex crucial for fear conditioning, but the CEL also appears to be crucial. The CEL is downstream of the BLA but might also work in parallel to form fear memories because it also receives connections from auditory thalamic nuclei and cortical areas. Because the CEA is downstream of these structures, the CEA might be able to override stimulus discrimination established in upstream structures such as sensory and association cortex and thalamic regions.

Furthermore, feed forward inhibition from intercalated (ITC) neurons might implicate the CEL as the primary target for fear extinction. ITC cells are a very small subpopulation of neurons located just medial to the BLA complex, and they appear to be necessary for extinction. Selectively lesioning ITC neurons results in a marked impairment in extinction learning [93]. ITC neurons receive glutamatergic input from the PFC [94,95] and directly project to both the CEL and CEm [91]. Activating the infralimbic region of the PFC resulted in activation of the immediate early gene, *c-fos*, in ITC neurons [95], and extinction produced an excitation in ITC neurons, which resulted in inhibition of the CEA output neurons [95]. The BLA also synapses onto ITC neurons [96], providing another level of regulation of fear learning and extinction (Figure 4). Clearly, fear conditioning and extinction are under tight regulatory control by GABAergic signaling, and as will be discussed in the next section, glutamatergic signaling also plays a key regulatory role.

### Glutamatergic signaling in fear conditioning

Glutamate is the main excitatory neurotransmitter in the brain, therefore it is not surprising that glutamatergic signaling is essential for the consolidation and extinction of fear. Glutamatergic cells in the BLA are activated after fear conditioning in rodents [97]. The BLA receives glutamatergic input from the sensory thalamic and cortical structures as well as the hippocampus and PFC [35]. In addition, the BLA sends glutamatergic signals to the CEA, which regulates the inhibitory microcircuits reviewed in the previous section. Glutamate acts on a variety of ionotropic (NMDA, AMPA) and metabotropic receptors (mGluR 1–8), which have been widely demonstrated to play a role in fear conditioning. Ionotropic glutamate receptors are the key mediators of synaptic plasticity required for long-term fear memories, whereas mGluRs modulate synaptic plasticity through G-protein coupled signal transduction.



**Figure 4.** Schematic diagram illustrating the key amygdala nuclei involved in fear conditioning. Microcircuits within the amygdala demonstrate multiple levels of regulation with response to fear consolidation, extinction and the expression of fear. Initially, it was thought that the basolateral nucleus of the amygdala (BLA) complex was solely responsible for fear acquisition and was the main recipient of thalamic and cortical inputs. The central amygdala was thought to be crucial only for the expression of conditioned fear responses via activation of downstream neural structures [35,151]. Now, significant evidence supports the idea that the lateral division of the central amygdala (CEM) is also critical for acquisition of fear and also receives cortical and thalamic inputs. In addition, intercalated neurons might regulate firing of central amygdala output neurons and the expression of extinction. The intercalated neurons receive projections from the infralimbic cortex (a region critical for extinction) and project GABAergic inhibitory neurons onto the medial division of the central amygdala (CEM).

Fear conditioning appears to result in an activation of NMDA receptors [98]. There are multiple ways by which NMDA activation contributes to synaptic plasticity in the amygdala, some of which are described below. Fear conditioning also results in NMDA receptor-dependent increases in degradation-specific polyubiquitination in the amygdala, targeting proteins involved in translational control and synaptic structure [99]. This recent study also showed that blocking the degradation of these proteins significantly impairs long-term memory. In addition to these mechanisms, within the synapse downstream signaling mechanisms result in a subsequent insertion of additional AMPA receptors at synaptic sites [98–103]. This increase in surface AMPA receptors results in LTP and an increased responsiveness of the synapse to future CS+ presentations. Antagonizing NMDA receptors in either the hippocampus or BLA impairs consolidation of fear conditioning [104–106]. Blocking AMPA receptor insertion in the synaptic membrane in the lateral amygdala blocks fear memory formation [101,102]. Extinction of fear conditioning also appears to be regulated by NMDA and AMPA receptor signaling. Antagonizing NMDA receptors can impair extinction in rodents [106,107]. In addition, there appears to be a reduction in surface AMPA receptors after extinction, relative to fear-conditioned animals that were not extinguished [108].

Changes in NMDA/AMPA ratios appear to happen rapidly during consolidation of memory, but the question remains: how is glutamatergic signaling translated into a long-term memory and how is that memory biologically maintained? Protein kinase M zeta (PKM $\zeta$ ) is an atypical isoform of PKC that can stay chronically active despite molecular turnover. Overexpression of PKM $\zeta$  enhances long-term memory [109] and inhibiting PKM $\zeta$  can disrupt memory, even after that memory has been formed [109–114]. In addition, PKM $\zeta$  inactivation-induced impairment of fear memory appears to correlate with a decrease in expression of the GluR2 subunit of the AMPA receptor [110]. Furthermore, blocking GluR2-dependent removal of postsynaptic AMPA receptors abolished behavioral

impairment of PKM $\zeta$  inhibition [110], suggesting that PKM $\zeta$  might be a mechanistic switch that maintains memory over time through the regulation of AMPA receptor trafficking. However, a pharmacological inhibitor of PKM $\zeta$  only temporarily disrupts expression of fear conditioning when administered to rats immediately prior to testing and does not completely abolish the fear memory [111]. Thus, at least based on these findings, it appears that PKM $\zeta$  is an unlikely drug target for PTSD.

An alternative promising avenue for the modulation of glutamatergic signaling has been the development of D-cycloserine (DCS), an NMDA partial agonist. DCS has been shown to facilitate extinction learning in animals and humans [115–127]. More recently, DCS has been suggested to reverse the reduction in AMPA receptors that is normally observed at synaptic sites in the lateral amygdala after fear learning [97]. Clinically, DCS has been shown to be a valuable augmentation to behavioral therapies for a variety of anxiety-related disorders, including obsessive-compulsive disorder [121–125,127,128], however definitive trials specifically for PTSD treatment using DCS have yet to be completed. DCS is an example of a drug that enhances the extinction of fear in animals and humans, as well as enhancing behavioral therapy in individuals with anxiety disorders involving fear dysregulation.

mGluRs modulate synaptic plasticity in the brain and are critical for the consolidation of fear conditioning and extinction. Although there have been mixed reports about the effect of mGluR agonists on fear conditioning, in general, mGluR antagonists and genetic deletion of mGluRs in the limbic regions of the brain appear to impair both consolidation and extinction of fear conditioning [129–134]. Activation of mGluR1-containing receptors in the BLA is known to enhance fear learning [135].

Many other receptor–ligand systems play a modulatory role in Pavlovian fear conditioning and probably contribute to PTSD, mostly by modulating GABAergic and glutamatergic signaling (Table 1). Two retrograde signaling systems (involving nitric oxide and endocannabinoids as the

**Table 1. Other ligand–receptor systems involved in the regulation of Pavlovian fear conditioning**

System	Function	Supporting evidence <sup>a</sup>	Refs
Norepinephrine (NE)	Consolidation	Enhanced with $\alpha_1$ -adrenergic receptor antagonists	[142]
	Extinction	Impaired by siRNA for $\beta_1$ -adrenergic receptors	[143]
NOS-cGMP	Consolidation	Impaired by antagonizing NE receptors in the infralimbic cortex	[144,145]
Endocannabinoid	Consolidation	Enhanced by PKG activation in the LA	[137]
		Impaired contextual conditioning in nNOS KO mice	[138]
		Impaired in cGMP mutant mice	[139]
	Extinction	Impaired by NOS and PKG inhibition in the LA	[140]
Dopamine (DA)	Consolidation	CB1 mRNA increases 48 h after fear conditioning	[136]
		Enhanced by inverse agonist of CB1 in the CEA or BLA	[136]
		Impaired by CB1 receptor agonist or AEA transport inhibition into the vmPFC	[136]
	Extinction	Impaired by pharmacological blockade or genetic deletion of CB1 receptors	[141,152]
Acetylcholine (Ach)	Consolidation	Enhanced by D2 receptor agonists in the VTA	[146,147]
		D2 receptor antagonists in the BLA impair fear potentiated startle	[146]
	Extinction	Impaired by D1 receptor loss (genetic KO or siRNA in hippocampus)	[148]
		Impaired by systemic or intra-IL PFC infusion of D2 antagonist	[149]
	Consolidation	Enhanced by nicotinic Ach (nACh) agonists in the hippocampus	[153–155]
		Impaired by $\alpha_7$ nACh receptor antagonists	[156]
	Extinction	Impaired by nACh agonists	[157]

<sup>a</sup>Abbreviations: AEA, anandamide; CB1, cannabinoid receptor type 1; IL, infralimbic; KO, knockout; NOS, nitric oxide synthase; PKG, cGMP-dependent protein kinase; siRNA, small interfering RNA.

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**Box 2. Outstanding questions**

- Why are some individuals at risk for developing PTSD, but despite similar trauma, others appear to be resistant?
- Furthermore, as with many common diseases, PTSD will probably represent a final common pathway of a 'broken brain' at the intersection of trauma and biology. How many different 'subtypes' of PTSD might there be?
- Will our current syndromal nomenclature be predictive of these subtypes, or will future biomarkers provide new ways of dissecting this syndrome?
- Is the resilience that we define as lack of PTSD, despite severe trauma, simply the absence of PTSD symptoms (along with comorbid depression and substance abuse) or is resilience an orthogonal construct that is uniquely protective?
- Up to 30–35% of risk for PTSD appears to be heritable [163]. Similar to a number of other disorders, will this be made up of many common gene variants, which each contribute only a small percentage of risk, or will there be a larger number of rare variants that each contribute higher levels of risk?
- With sufficient trauma loading, almost anyone is susceptible to PTSD. Genes appear to differentially modulate the level of susceptibility at a given trauma level or trauma 'dose'. How do the effects of childhood and adult trauma interact through neural circuitry with genes that contribute risk, and which might act in an additive fashion on this same circuitry?
- The neural circuitry modulating fear, including the amygdala, PFC and hippocampal regions are conserved across mammals. This makes research on PTSD and other anxiety-related disorders more readily accessible to translation compared to many other mental disorders. Utilizing human dynamic and structural neuroimaging techniques combined with rodent and other laboratory model species, we can ask how do these different regions that organize and modulate the emotion of fear work in concert?

retrograde messengers) have been shown to be important for presynaptically-regulated plasticity in consolidation and extinction, respectively [136–141]. Noradrenergic signaling from the locus coeruleus [142–145], and dopaminergic projections to the amygdala from the ventral tegmental area (VTA) and nucleus accumbens [146–149] also play important roles in modulating synaptic plasticity and fear conditioning. These transmitter systems could provide additional potential molecular targets for the pharmacological augmentation of behavioral therapy for PTSD.

**Concluding remarks**

The molecular pathways discussed in this review are crucial for fear conditioning and extinction. Recent research has advanced our understanding of many of the downstream molecular mechanisms of these forms of learning. By understanding the genetics of PTSD we might eventually be better able to predict which individuals might be more susceptible to developing PTSD after trauma. In addition, knowing the molecular underpinnings of PTSD will provide important new insights into molecular targets for drug development. By generating drugs that modulate signaling pathways involved in fear conditioning and synaptic plasticity in the amygdala, we might be able to enhance extinction of inappropriate fear associations or even prevent the development of fear associations in individuals more susceptible to PTSD. Research in this area shows great promise for potential new approaches to better understand the physiology of circuits mediating fear responses, as well as to potentially further the prevention and treatment of PTSD (Box 2). Given the rising numbers

of traumatized civilians and veterans, in addition to our increasing understanding of the prevalence, comorbidity and sequelae of PTSD, developing better preventions and treatments are vital.

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