

Special Issue: Neuropsychiatric Disorders

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

Amy L. Mahan¹ and Kerry J. Ressler^{1,2}¹ Center for Behavioral Neuroscience, Department of Psychiatry and Behavioral Sciences, Yerkes National Primate Research Center, Emory University School of Medicine, 954 Gatewood Drive, Atlanta, GA 30329, USA² Howard Hughes Medical Institute, Chevy Chase, MD, USA

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

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). ***

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

Corresponding author: Ressler, K.J. (kressle@emory.edu).

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.

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].

Serotonin transporter (SERT): short versus long allele

Function: serotonin transport/reuptake

Result of polymorphism:

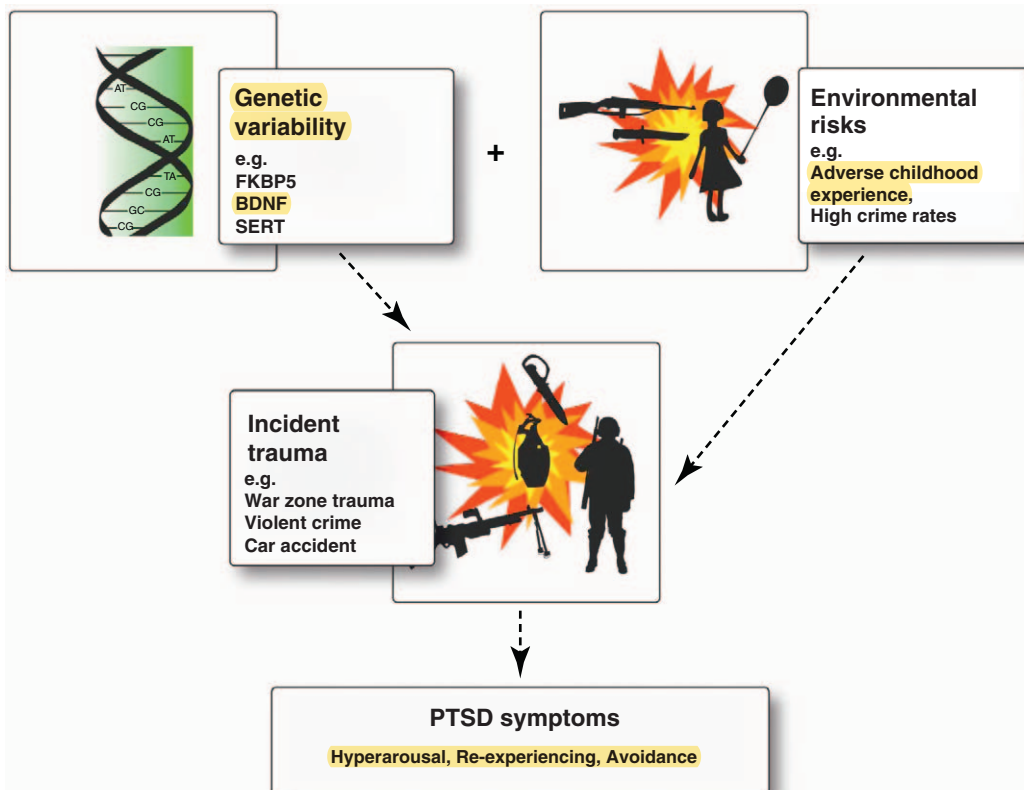
- Different alleles have been associated with altered SERT gene expression/translation [158–160].
- Findings have been reported in individuals for an increased risk of PTSD with both the long [158,159] and short allele [158,160].
- Recent data suggest that the short allele is associated with decreased 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 neuropsin is critical for stress-related plasticity in the amygdala by regulating EphB2-NMDA-receptor activation of FKBP5 expression [162].



TRENDS in Neurosciences

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

PTSD = aberrant (abnormal) synaptic plasticity (= prolonged synaptic activity)

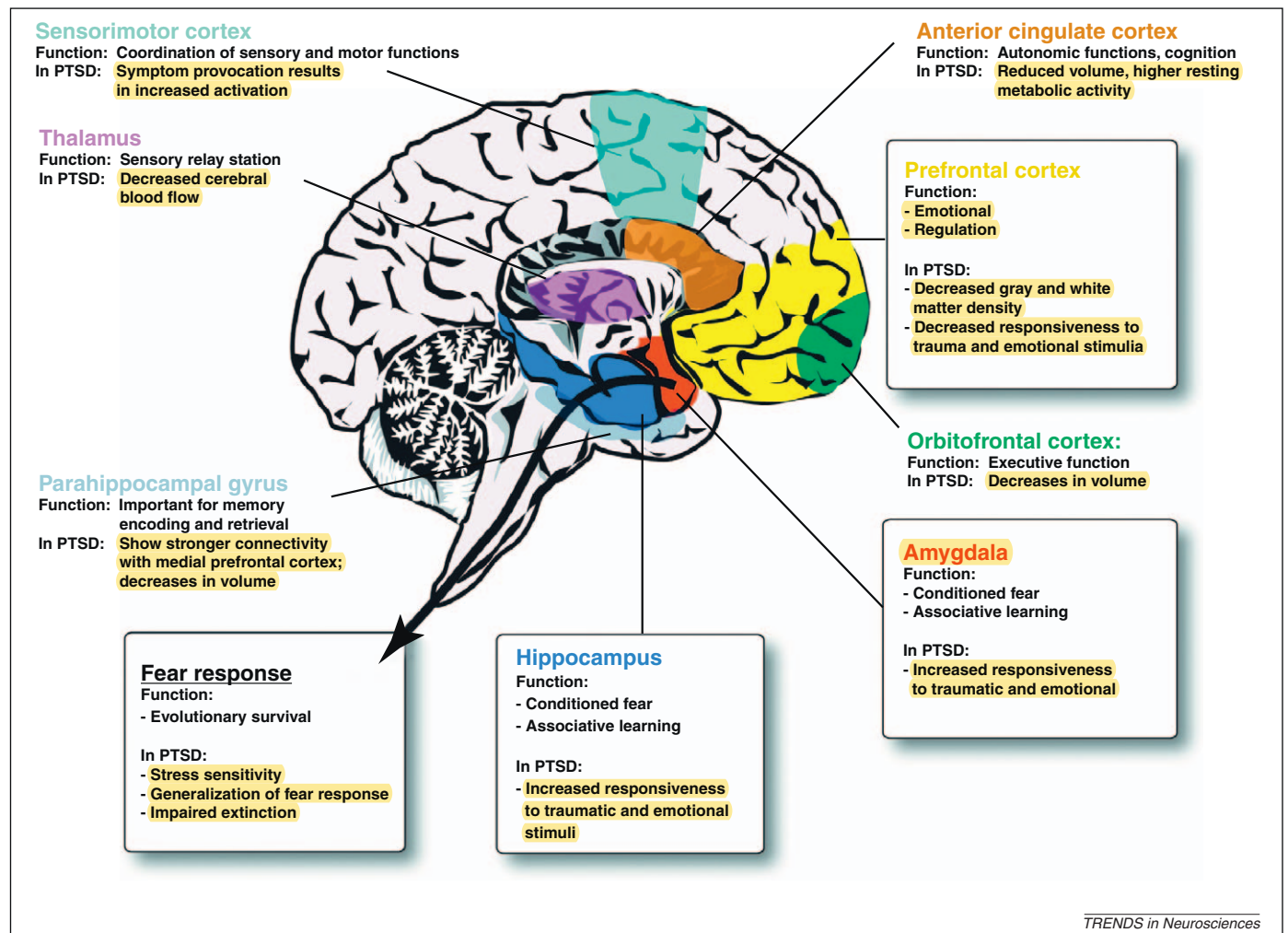


Figure 1. A schematic of the human brain illustrating how the limbic system is involved in posttraumatic stress disorder (PTSD). The prefrontal cortex (PFC) and the hippocampus both have dense connections to the amygdala, which is important for conditioned fear and associative emotional learning. The 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].

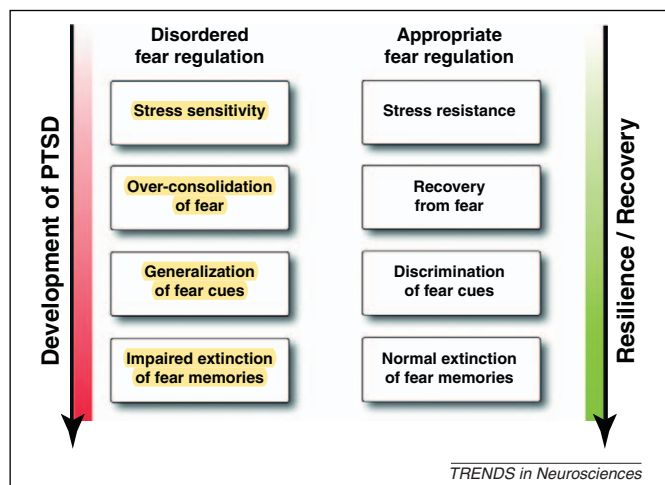


Figure 2. Disordered fear regulation in posttraumatic stress disorder (PTSD). Individuals with PTSD typically show increased sensitization to stress, over-generalization of fear to irrelevant stimuli and impaired extinction of fear memories. Individuals who demonstrate resilience to PTSD, and/or who 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.

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 insight 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) ***
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/)

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

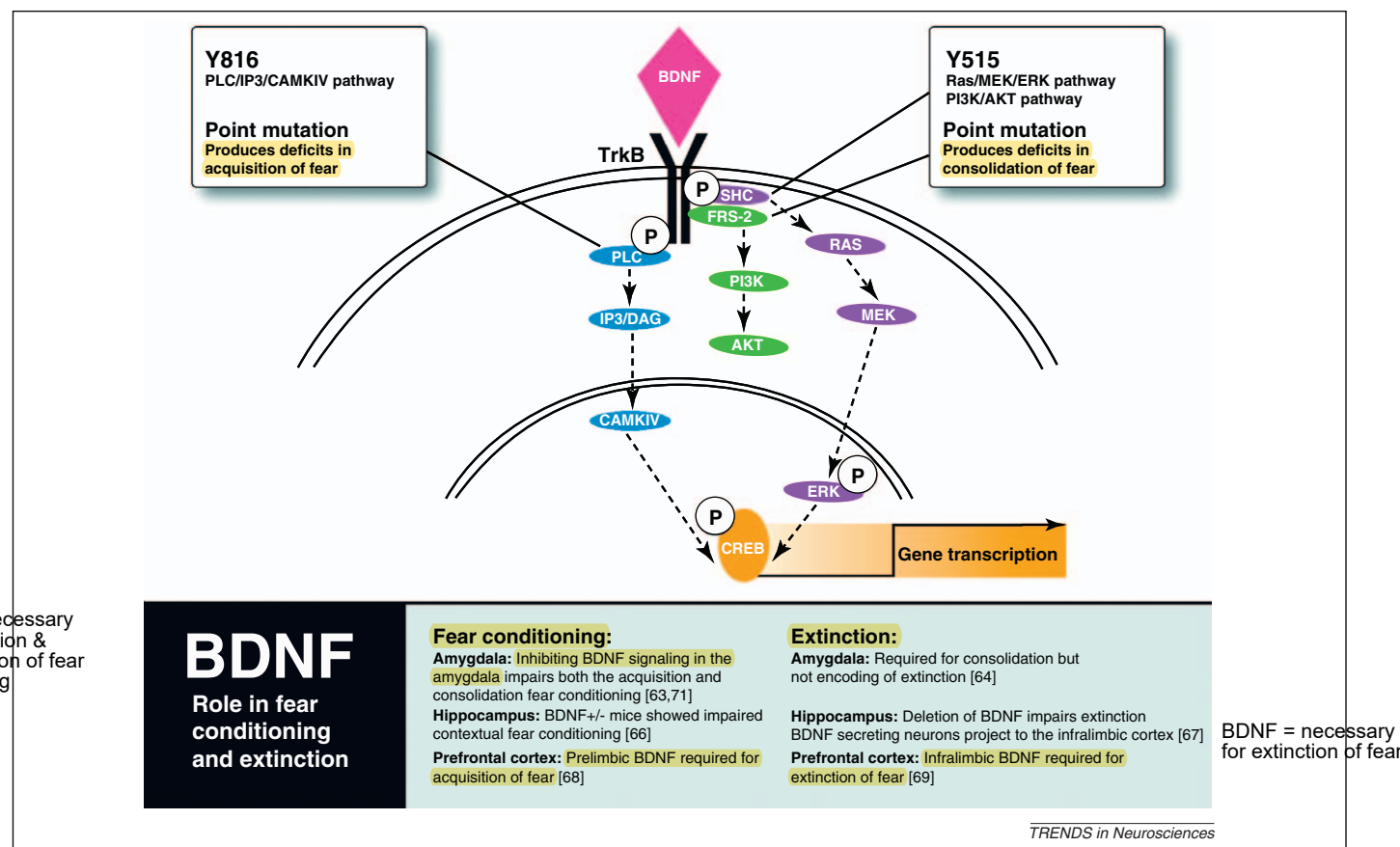


Figure 3. The brain-derived neurotrophic factor (BDNF)–tyrosine kinase B (TrkB) induced signaling pathway. BDNF binds to the TrkB receptor, resulting in the phosphorylation of two tyrosine sites (Y515 and Y816) on the intracellular domain of the TrkB receptor. Phosphorylation of the Y515 residue allows 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 Ca²⁺/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 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.

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_A 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.

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 CEI 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 CEI [90]. These neuronal subpopulations were termed CEI 'on' and 'off' neurons based on their response to fear conditioning. CEI 'on' neurons acquired an excitatory response to the CS+ during and after fear acquisition, whereas CEI 'off' neurons showed decreased responses to the CS+ during and after fear acquisition. CS evoked excitation of CEI 'on' neurons began before the CEI 'off' neurons, and

Fear -->
reduces
GABAergic
signaling

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/>

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 δ isoform of protein kinase C (PKC- δ) [91]. Using molecular and genetic approaches, this study was able to map the functional connectivity of PKC- δ + and PKC- δ - neurons. Specifically, optogenetic targeting was employed to examine the effect of reversibly silencing PKC- δ + neurons on the activity of CEL 'on', CEL 'off' and CEm neurons. PKC- δ + neurons were found to be predominantly late firing neurons, which reciprocally inhibit PKC- δ - neurons. Inactivation of PKC- δ + 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- δ + neurons. Taken together, these findings suggest that the PKC- δ + 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.

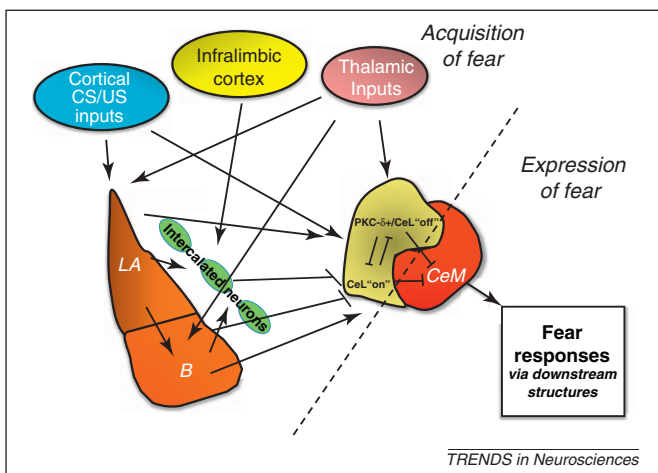


Figure 4. Schematic diagram illustrating the key amygdala nuclei involved in fear conditioning. Microcircuits within the amygdala demonstrate multiple levels of regulation with response to fear consolidation, extinction and the expression of fear. Initially, it was thought that the basolateral nucleus 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 (CEL) 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 ζ) is an atypical isoform of PKC that can stay chronically active despite molecular turnover. Overexpression of PKM ζ enhances long-term memory [109] and inhibiting PKM ζ can disrupt memory, even after that memory has been formed [109–114]. In addition, PKM ζ 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 ζ inhibition [110], suggesting that PKM ζ might be a mechanistic switch that maintains memory over time through the regulation of AMPA receptor trafficking. However, a pharmacological inhibitor of PKM ζ 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 ζ 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 ^a	Refs
Norepinephrine (NE)	Consolidation	Enhanced with α_1 -adrenergic receptor antagonists	[142]
		Impaired by siRNA for β_1 -adrenergic receptors	[143]
	Extinction	Impaired by antagonizing NE receptors in the infralimbic cortex	[144,145]
NOS-cGMP	Consolidation	Enhanced by PKG activation in the LA	[137]
		Impaired contextual conditioning in nNOS KO mice	[138]
		Impaired in cGMP mutant mice	[139]
		Impaired by NOS and PKG inhibition in the LA	[140]
Endocannabinoid	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]
Dopamine (DA)	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]
Acetylcholine (ACh)	Consolidation	Enhanced by nicotinic ACh (nACh) agonists in the hippocampus	[153–155]
		Impaired by α_7 nACh receptor antagonists	[156]
	Extinction	Impaired by nACh agonists	[157]

^aAbbreviations: AEA, anandamide; CB1, cannabinoid receptor type 1; IL, infralimbic; KO, knockout; NOS, nitric oxide synthase; PKG, cGMP-dependent protein kinase; siRNA, small interfering RNA.

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 'sub-types' 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.

Acknowledgments

Support was provided by the National Institutes of Health (MH071537, DA019624 and MH086189), the Burroughs Wellcome Fund and the National Primate Research Center base grant #RR-001665. We would like to thank Jennifer L. Williams for help in design of the figures in this manuscript.

References

- 1 Gillespie, C.F. *et al.* (2009) Trauma exposure and stress-related disorders in inner city primary care patients. *Gen. Hosp. Psychiatry* 31, 505–514
- 2 American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders*, American Psychiatric Association
- 3 Milliken, C.S. *et al.* (2007) Longitudinal assessment of mental health problems among active and reserve component soldiers returning from the Iraq war. *JAMA* 298, 2141–2148
- 4 Davidson, J.R. *et al.* (2004) Posttraumatic stress disorder: acquisition, recognition, course, and treatment. *J. Neuropsychiatry Clin. Neurosci.* 16, 135–147
- 5 Hoge, C.W. *et al.* (2007) Association of posttraumatic stress disorder with somatic symptoms, health care visits, and absenteeism among Iraq war veterans. *Am. J. Psychiatry* 164, 150–153
- 6 Wilcox, H.C. *et al.* (2009) Posttraumatic stress disorder and suicide attempts in a community sample of urban american young adults. *Arch. Gen. Psychiatry* 66, 305–311
- 7 Lanius, R.A. *et al.* (2006) A review of neuroimaging studies in PTSD: heterogeneity of response to symptom provocation. *J. Psychiatr. Res.* 40, 709–729
- 8 Dickie, E.W. *et al.* (2008) An fMRI investigation of memory encoding in PTSD: influence of symptom severity. *Neuropsychologia* 46, 1522–1531
- 9 Binder, E.B. *et al.* (2008) Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* 299, 1291–1305
- 10 Bradley, R.G. *et al.* (2008) Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor gene. *Arch. Gen. Psychiatry* 65, 190–200
- 11 Green, K.T. *et al.* (2010) Exploration of the resilience construct in posttraumatic stress disorder severity and functional correlates in military combat veterans who have served since September 11, 2001. *J. Clin. Psychiatry* 71, 823–830
- 12 Jovanovic, T. and Ressler, K.J. (2010) How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *Am. J. Psychiatry* 167, 648–662
- 13 Heimer, L. and Van Hoesen, G.W. (2006) The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neurosci. Biobehav. Rev.* 30, 126–147
- 14 Etkin, A. and Wager, T.D. (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am. J. Psychiatry* 164, 1476–1488
- 15 Francati, V. *et al.* (2007) Functional neuroimaging studies in posttraumatic stress disorder: review of current methods and findings. *Depress. Anxiety* 24, 202–218
- 16 Quirk, G.J. and Mueller, D. (2008) Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* 33, 56–72
- 17 van Marle, H.J. *et al.* (2009) From specificity to sensitivity: how acute stress affects amygdala processing of biologically salient stimuli. *Biol. Psychiatry* 66, 649–655
- 18 de Carvalho, M.R. *et al.* (2010) The fear circuitry in panic disorder and its modulation by cognitive-behaviour therapy interventions. *World J. Biol. Psychiatry* 11, 188–198
- 19 Abe, O. *et al.* (2006) Voxel-based diffusion tensor analysis reveals aberrant anterior cingulum integrity in posttraumatic stress disorder due to terrorism. *Psychiatry Res.* 146, 231–242
- 20 Thomaes, K. *et al.* (2010) Reduced anterior cingulate and orbitofrontal volumes in child abuse-related complex PTSD. *J. Clin. Psychiatry* 71, 1636–1644

- 21 Rogers, M.A. *et al.* (2009) Smaller amygdala volume and reduced anterior cingulate gray matter density associated with history of post-traumatic stress disorder. *Psychiatry Res.* 174, 210–216
- 22 McLaughlin, K.A. *et al.* (2010) Childhood adversity, adult stressful life events, and risk of past-year psychiatric disorder: a test of the stress sensitization hypothesis in a population-based sample of adults. *Psychol. Med.* 40, 1647–1658
- 23 Griffin, M.G. (2008) A prospective assessment of auditory startle alterations in rape and physical assault survivors. *J. Trauma Stress* 21, 91–99
- 24 Ehlers, A. *et al.* (2010) Heart rate responses to standardized trauma-related pictures in acute posttraumatic stress disorder. *Int. J. Psychophysiol.* 78, 27–34
- 25 Pole, N. *et al.* (2009) Prospective prediction of posttraumatic stress disorder symptoms using fear potentiated auditory startle responses. *Biol. Psychiatry* 65, 235–240
- 26 Suendermann, O. *et al.* (2010) Early heart rate responses to standardized trauma-related pictures predict posttraumatic stress disorder: a prospective study. *Psychosom. Med.* 72, 301–308
- 27 Milad, M.R. *et al.* (2008) Presence and acquired origin of reduced recall for fear extinction in PTSD: results of a twin study. *J. Psychiatr. Res.* 42, 515–520
- 28 Blechert, J. *et al.* (2007) Fear conditioning in posttraumatic stress disorder: evidence for delayed extinction of autonomic, experiential, and behavioural responses. *Behav. Res. Ther.* 45, 2019–2033
- 29 Wessa, M. and Flor, H. (2007) Failure of extinction of fear responses in posttraumatic stress disorder: evidence from second-order conditioning. *Am. J. Psychiatry* 164, 1684–1692
- 30 Shin, L.M. and Handwerker, K. (2009) Is posttraumatic stress disorder a stress-induced fear circuitry disorder? *J. Trauma Stress* 22, 409–415
- 31 Jovanovic, T. *et al.* (2009) Posttraumatic stress disorder may be associated with impaired fear inhibition: relation to symptom severity. *Psychiatry Res.* 167, 151–160
- 32 Yehuda, R. and LeDoux, J. (2007) Response variation following trauma: a translational neuroscience approach to understanding PTSD. *Neuron* 56, 19–32
- 33 Lang, P.J. *et al.* (2000) Fear and anxiety: animal models and human cognitive psychophysiology. *J. Affect. Disord.* 61, 137–159
- 34 Belzung, C. and Philippot, P. (2007) Anxiety from a phylogenetic perspective: is there a qualitative difference between human and animal anxiety? *Neural Plast.* 2007, 59676
- 35 Pape, H.C. and Pare, D. (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. *Physiol. Rev.* 90, 419–463
- 36 Sah, P. *et al.* (2008) Fear conditioning and long-term potentiation in the amygdala: what really is the connection? *Ann. N.Y. Acad. Sci.* 1129, 88–95
- 37 Sigurdsson, T. *et al.* (2007) Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. *Neuropharmacology* 52, 215–227
- 38 Maren, S. (2005) Synaptic mechanisms of associative memory in the amygdala. *Neuron* 47, 783–786
- 39 Blair, H.T. *et al.* (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn. Mem.* 8, 229–242
- 40 Quirk, G.J. *et al.* (1995) Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron* 15, 1029–1039
- 41 Rogan, M.T. *et al.* (1997) Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* 390, 604–607
- 42 McKernan, M.G. and Shinnick-Gallagher, P. (1997) Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature* 390, 607–611
- 43 Tsvetkov, E. *et al.* (2002) Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala. *Neuron* 34, 289–300
- 44 Shumyatsky, G.P. *et al.* (2005) stathmin, a gene enriched in the amygdala, controls both learned and innate fear. *Cell* 123, 697–709
- 45 Howland, J.G. and Wang, Y.T. (2008) Synaptic plasticity in learning and memory: stress effects in the hippocampus. *Prog. Brain Res.* 169, 145–158
- 46 Dell'osso, L. *et al.* (2009) Brain-derived neurotrophic factor plasma levels in patients suffering from post-traumatic stress disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 899–902
- 47 Hauck, S. *et al.* (2010) Serum brain-derived neurotrophic factor in patients with trauma psychopathology. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 459–462
- 48 Berger, W. *et al.* (2010) Serum brain-derived neurotrophic factor predicts responses to escitalopram in chronic posttraumatic stress disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 34, 1279–1284
- 49 Zhang, H. *et al.* (2006) Brain derived neurotrophic factor (BDNF) gene variants and Alzheimer's disease, affective disorders, posttraumatic stress disorder, schizophrenia, and substance dependence. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 141B, 387–393
- 50 Gonul, A.S. *et al.* (2011) Association of the brain-derived neurotrophic factor Val66Met polymorphism with hippocampus volumes in drug-free depressed patients. *World J. Biol. Psychiatry* 12, 110–118
- 51 Egan, M.F. *et al.* (2003) The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112, 257–269
- 52 Dennis, N.A. *et al.* (2010) Brain-derived neurotrophic factor val66met polymorphism and hippocampal activation during episodic encoding and retrieval tasks. *Hippocampus* DOI: 10.1002/hipo.20809
- 53 van Wingen, G. *et al.* (2010) The brain-derived neurotrophic factor Val66Met polymorphism affects memory formation and retrieval of biologically salient stimuli. *Neuroimage* 50, 1212–1218
- 54 Lonsdorf, T.B. *et al.* (2010) Amygdala-dependent fear conditioning in humans is modulated by the BDNF val66met polymorphism. *Behav. Neurosci.* 124, 9–15
- 55 Hajcak, G. *et al.* (2009) Genetic variation in brain-derived neurotrophic factor and human fear conditioning. *Genes Brain Behav.* 8, 80–85
- 56 Soliman, F. *et al.* (2010) A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science* 327, 863–866
- 57 Li, W.J. *et al.* (2010) Anxiolytic effect of music exposure on BDNF Met/Met transgenic mice. *Brain Res.* 1347, 71–79
- 58 Chen, Z.Y. *et al.* (2006) Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science* 314, 140–143
- 59 Yu, H. *et al.* (2009) Variant BDNF Val66Met polymorphism affects extinction of conditioned aversive memory. *J. Neurosci.* 29, 4056–4064
- 60 Ninan, I. *et al.* (2010) The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. *J. Neurosci.* 30, 8866–8870
- 61 Jang, S.W. *et al.* (2010) A selective TrkB agonist with potent neurotrophic activities by 7,8-dihydroxyflavone. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2687–2692
- 62 Heldt, S.A. *et al.* (2007) Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories. *Mol. Psychiatry* 12, 656–670
- 63 Takei, S. *et al.* (2011) Enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in an animal model of posttraumatic stress disorder. *J. Psychiatr. Res.* 45, 460–468
- 64 Yee, B.K. *et al.* (2007) Levels of neurotrophic factors in the hippocampus and amygdala correlate with anxiety- and fear-related behaviour in C57BL/6 mice. *J. Neural. Transm.* 114, 431–444
- 65 Rattiner, L.M. *et al.* (2004) Differential regulation of brain-derived neurotrophic factor transcripts during the consolidation of fear learning. *Learn. Mem.* 11, 727–731
- 66 Chhatwal, J.P. *et al.* (2006) Amygdala BDNF signaling is required for consolidation but not encoding of extinction. *Nat. Neurosci.* 9, 870–872
- 67 Rattiner, L.M. *et al.* (2004) Brain-derived neurotrophic factor and tyrosine kinase receptor B involvement in amygdala-dependent fear conditioning. *J. Neurosci.* 24, 4796–4806
- 68 Ou, L.C. *et al.* (2010) Late expression of brain-derived neurotrophic factor in the amygdala is required for persistence of fear memory. *Neurobiol. Learn. Mem.* 93, 372–382
- 69 Liu, I.Y. *et al.* (2004) Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning. *J. Neurosci.* 24, 7958–7963
- 70 Choi, D.C. *et al.* (2010) Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. *Proc. Natl. Acad. Sci. U.S.A.* 107, 2675–2680

- 71 Peters, J. *et al.* (2010) Induction of fear extinction with hippocampal-infralimbic BDNF. *Science* 328, 1288–1290
- 72 Musumeci, G. *et al.* (2009) TrkB modulates fear learning and amygdalar synaptic plasticity by specific docking sites. *J. Neurosci.* 29, 10131–10143
- 73 Andero, R. *et al.* (2011) Effect of 7,8-dihydroxyflavone, a small-molecule TrkB agonist, on emotional learning. *Am. J. Psychiatry* 168, 163–172
- 74 Li, C. *et al.* (2011) Synergistic activation of dopamine D1 and TrkB receptors mediate gain control of synaptic plasticity in the basolateral amygdala. *PLoS One* 6, e26065
- 75 Meis, S. *et al.* (2011) Postsynaptic BDNF signaling regulates long-term potentiation at thalamo-amygdala afferents. *J. Physiol.* DOI:10.1113/jphysiol.2011.220434
- 76 Ou, L.C. and Gean, P.W. (2006) Regulation of amygdala-dependent learning by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol-3-kinase. *Neuropsychopharmacology* 31, 287–296
- 77 Ressler, K.J. *et al.* (2011) Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 470, 492–497
- 78 Monsey, M.S. *et al.* (2011) Epigenetic alterations are critical for fear memory consolidation and synaptic plasticity in the lateral amygdala. *PLoS One* 6, e19958
- 79 Tolin, D.F. and Foa, E.B. (2006) Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. *Psychol. Bull.* 132, 959–992
- 80 Makkar, S.R. *et al.* (2010) Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. *Neuropsychopharmacology* 35, 1625–1652
- 81 Bolshakov, V.Y. (2009) Nipping fear in the bud: inhibitory control in the amygdala. *Neuron* 61, 817–819
- 82 Zhang, S. and Cranney, J. (2008) The role of GABA and anxiety in the reconsolidation of conditioned fear. *Behav. Neurosci.* 122, 1295–1305
- 83 Rea, K. *et al.* (2009) Alterations in extracellular levels of gamma-aminobutyric acid in the rat basolateral amygdala and periaqueductal gray during conditioned fear, persistent pain and fear-conditioned analgesia. *J. Pain* 10, 1088–1098
- 84 Wiltgen, B.J. *et al.* (2009) The alpha1 subunit of the GABA(A) receptor modulates fear learning and plasticity in the lateral amygdala. *Front. Behav. Neurosci.* 3, 37
- 85 Raybuck, J.D. and Lattal, K.M. (2011) Double dissociation of amygdala and hippocampal contributions to trace and delay fear conditioning. *PLoS ONE* 6, e15982
- 86 Sierra-Mercado, D. *et al.* (2011) Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 36, 529–538
- 87 Corbit, L.H. and Janak, P.H. (2010) Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. *Eur. J. Neurosci.* 31, 1312–1321
- 88 Hart, G. *et al.* (2010) Systemic or intra-amygdala infusion of the benzodiazepine, midazolam, impairs learning, but facilitates re-learning to inhibit fear responses in extinction. *Learn. Mem.* 17, 210–220
- 89 Laurent, V. and Westbrook, R.F. (2010) Role of the basolateral amygdala in the reinstatement and extinction of fear responses to a previously extinguished conditioned stimulus. *Learn. Mem.* 17, 86–96
- 90 Ciochi, S. *et al.* (2010) Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature* 468, 277–282
- 91 Haubensak, W. *et al.* (2010) Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature* 468, 270–276
- 92 Tye, K.M. *et al.* (2011) Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature* 471, 358–362
- 93 Likhtik, E. *et al.* (2008) Amygdala intercalated neurons are required for expression of fear extinction. *Nature* 454, 642–645
- 94 Amano, T. *et al.* (2010) Synaptic correlates of fear extinction in the amygdala. *Nat. Neurosci.* 13, 489–494
- 95 Berretta, S. *et al.* (2005) Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience* 132, 943–953
- 96 Izumi, T. *et al.* (2011) Retrieval of conditioned fear activates the basolateral and intercalated nucleus of amygdala. *J. Neurosci. Res.* 89, 773–790
- 97 Lin, H.C. *et al.* (2010) Alterations of excitatory transmission in the lateral amygdala during expression and extinction of fear memory. *Int. J. Neuropsychopharmacol.* 13, 335–345
- 98 Nedelescu, H. *et al.* (2010) Endogenous GluR1-containing AMPA receptors translocate to asymmetric synapses in the lateral amygdala during the early phase of fear memory formation: an electron microscopic immunocytochemical study. *J. Comp. Neurol.* 518, 4723–4739
- 99 Jarome, T.J. (2011) Activity dependent protein degradation is critical for the formation and stability of fear memory in the amygdala. *PLoS One* 6, e24349
- 100 Liu, Y. *et al.* (2010) A single fear-inducing stimulus induces a transcription-dependent switch in synaptic AMPAR phenotype. *Nat. Neurosci.* 13, 223–231
- 101 Mokin, M. *et al.* (2007) Conversion of silent synapses into the active pool by selective GluR1-3 and GluR4 AMPAR trafficking during in vitro classical conditioning. *J. Neurophysiol.* 98, 1278–1286
- 102 Rumpel, S. *et al.* (2005) Postsynaptic receptor trafficking underlying a form of associative learning. *Science* 308, 83–88
- 103 Brigman, J.L. *et al.* (2010) Loss of GluN2B-containing NMDA receptors in CA1 hippocampus and cortex impairs long-term depression, reduces dendritic spine density, and disrupts learning. *J. Neurosci.* 30, 4590–4600
- 104 Zimmerman, J.M. and Maren, S. (2010) NMDA receptor antagonism in the basolateral but not central amygdala blocks the extinction of Pavlovian fear conditioning in rats. *Eur. J. Neurosci.* 31, 1664–1670
- 105 Dalton, G.L. *et al.* (2008) Disruption of AMPA receptor endocytosis impairs the extinction, but not acquisition of learned fear. *Neuropsychopharmacology* 33, 2416–2426
- 106 Liu, J.L. *et al.* (2009) A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory conditioned fear responses in a Pavlovian model. *PLoS ONE* 4, e7548
- 107 Falls, W.A. *et al.* (1992) Extinction of fear-potentiated startle: blockade by infusion of an NMDA antagonist into the amygdala. *J. Neurosci.* 12, 854–863
- 108 Clem, R.L. and Huganir, R.L. (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* 330, 1108–1112
- 109 Hardt, O. *et al.* (2010) PKMzeta maintains 1-day- and 6-day-old long-term object location but not object identity memory in dorsal hippocampus. *Hippocampus* 20, 691–695
- 110 Miguez, P.V. *et al.* (2010) PKMzeta maintains memories by regulating GluR2-dependent AMPA receptor trafficking. *Nat. Neurosci.* 13, 630–634
- 111 Parsons, R.G. and Davis, M. (2011) Temporary disruption of fear-potentiated startle following PKMzeta inhibition in the amygdala. *Nat. Neurosci.* 14, 295–296
- 112 Cohen, H. *et al.* (2010) Mapping the brain pathways of traumatic memory: inactivation of protein kinase M zeta in different brain regions disrupts traumatic memory processes and attenuates traumatic stress responses in rats. *Eur. Neuropsychopharmacol.* 20, 253–271
- 113 Kwapis, J.L. *et al.* (2009) Protein kinase Mzeta maintains fear memory in the amygdala but not in the hippocampus. *Behav. Neurosci.* 123, 844–850
- 114 Serrano, P. *et al.* (2008) PKMzeta maintains spatial, instrumental, and classically conditioned long-term memories. *PLoS Biol.* 6, 2698–2706
- 115 Guastella, A.J. *et al.* (2007) A randomized controlled trial of the effect of D-cycloserine on exposure therapy for spider fear. *J. Psychiatr. Res.* 41, 466–471
- 116 Langton, J.M. and Richardson, R. (2008) D-cycloserine facilitates extinction the first time but not the second time: an examination of the role of NMDA across the course of repeated extinction sessions. *Neuropsychopharmacology* 33, 3096–3102
- 117 McCallum, J. *et al.* (2010) Impaired extinction retention in adolescent rats: effects of D-cycloserine. *Neuropsychopharmacology* 35, 2134–2142
- 118 Kalisch, R. *et al.* (2009) The NMDA agonist D-cycloserine facilitates fear memory consolidation in humans. *Cereb. Cortex* 19, 187–196
- 119 Ledgerwood, L. *et al.* (2005) D-cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. *Biol. Psychiatry* 57, 841–847

- 120 Langton, J.M. and Richardson, R. (2009) The role of context in the re-extinction of learned fear. *Neurobiol. Learn. Mem.* 92, 496–503
- 121 Ressler, K.J. *et al.* (2004) Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Arch. Gen. Psychiatry* 61, 1136–1144
- 122 Guastella, A.J. *et al.* (2007) A randomized controlled trial of the effect of D-cycloserine on extinction and fear conditioning in humans. *Behav. Res. Ther.* 45, 663–672
- 123 Wilhelm, S. *et al.* (2008) Augmentation of behavior therapy with D-cycloserine for obsessive-compulsive disorder. *Am. J. Psychiatry* 165, 335–341 quiz 409
- 124 Kushner, M.G. *et al.* (2007) D-cycloserine augmented exposure therapy for obsessive-compulsive disorder. *Biol. Psychiatry* 62, 835–838
- 125 Norberg, M.M. *et al.* (2008) A meta-analysis of D-cycloserine and the facilitation of fear extinction and exposure therapy. *Biol. Psychiatry* 63, 1118–1126
- 126 Otto, M.W. *et al.* (2010) Efficacy of CBT for benzodiazepine discontinuation in patients with panic disorder: further evaluation. *Behav. Res. Ther.* 48, 720–727
- 127 Otto, M.W. *et al.* (2010) Efficacy of d-cycloserine for enhancing response to cognitive-behavior therapy for panic disorder. *Biol. Psychiatry* 67, 365–370
- 128 Guastella, A.J. *et al.* (2008) A randomized controlled trial of D-cycloserine enhancement of exposure therapy for social anxiety disorder. *Biol. Psychiatry* 63, 544–549
- 129 Goddyn, H. *et al.* (2008) Deficits in acquisition and extinction of conditioned responses in mGluR7 knockout mice. *Neurobiol. Learn. Mem.* 90, 103–111
- 130 More, L. *et al.* (2007) Comparison of the mGluR1 antagonist A-841720 in rat models of pain and cognition. *Behav. Pharmacol.* 18, 273–281
- 131 Kim, J. *et al.* (2007) Blockade of amygdala metabotropic glutamate receptor subtype 1 impairs fear extinction. *Biochem. Biophys. Res. Commun.* 355, 188–193
- 132 Fontanez-Nuin, D.E. *et al.* (2011) Memory for fear extinction requires mGluR5-mediated activation of infralimbic neurons. *Cereb. Cortex* 21, 727–735
- 133 Siegl, S. *et al.* (2008) Amygdaloid metabotropic glutamate receptor subtype 7 is involved in the acquisition of conditioned fear. *Neuroreport* 19, 1147–1150
- 134 Fendt, M. *et al.* (2010) The effect of mGlu8 deficiency in animal models of psychiatric diseases. *Genes Brain Behav.* 9, 33–44
- 135 Rudy, J.W. and Matus-Amat, P. (2009) DHPG activation of group 1 mGluRs in BLA enhances fear conditioning. *Learn. Mem.* 16, 421–425
- 136 Lisboa, S.F. *et al.* (2010) Cannabinoid CB1 receptors in the medial prefrontal cortex modulate the expression of contextual fear conditioning. *Int. J. Neuropsychopharmacol.* 13, 1163–1173
- 137 Ota, K.T. *et al.* (2010) Synaptic plasticity and NO-cGMP-PKG signaling regulate pre- and postsynaptic alterations at rat lateral amygdala synapses following fear conditioning. *PLoS ONE* 5, e11236
- 138 Kelley, J.B. *et al.* (2010) Pharmacological modulators of nitric oxide signaling and contextual fear conditioning in mice. *Psychopharmacology (Berl.)* 210, 65–74
- 139 Paul, C. *et al.* (2010) cGMP-dependent protein kinase type I promotes CREB/CRE-mediated gene expression in neurons of the lateral amygdala. *Neurosci. Lett.* 473, 82–86
- 140 Ota, K.T. *et al.* (2008) The NO-cGMP-PKG signaling pathway regulates synaptic plasticity and fear memory consolidation in the lateral amygdala via activation of ERK/MAP kinase. *Learn. Mem.* 15, 792–805
- 141 Chhatwal, J.P. *et al.* (2009) Functional interactions between endocannabinoid and CCK neurotransmitter systems may be critical for extinction learning. *Neuropsychopharmacology* 34, 509–521
- 142 Lazzaro, S.C. *et al.* (2010) Antagonism of lateral amygdala alpha1-adrenergic receptors facilitates fear conditioning and long-term potentiation. *Learn. Mem.* 17, 489–493
- 143 Fu, A.L. *et al.* (2007) Down-regulation of beta1-adrenoceptors gene expression by short interfering RNA impairs the memory retrieval in the basolateral amygdala of rats. *Neurosci. Lett.* 428, 77–81
- 144 Mueller, D. and Cahill, S.P. (2010) Noradrenergic modulation of extinction learning and exposure therapy. *Behav. Brain Res.* 208, 1–11
- 145 Mueller, D. *et al.* (2008) Noradrenergic signaling in infralimbic cortex increases cell excitability and strengthens memory for fear extinction. *J. Neurosci.* 28, 369–375
- 146 de Oliveira, A.R. *et al.* (2011) Conditioned fear is modulated by D2 receptor pathway connecting the ventral tegmental area and basolateral amygdala. *Neurobiol. Learn. Mem.* 95, 37–45
- 147 Biojone, C. *et al.* (2011) Anti-aversive effects of the atypical antipsychotic, aripiprazole, in animal models of anxiety. *J. Psychopharmacol.* 25, 801–807
- 148 Ortiz, O. *et al.* (2010) Associative learning and CA3-CA1 synaptic plasticity are impaired in D1R null, *Drd1a*^{−/−} mice and in hippocampal siRNA silenced *Drd1a* mice. *J. Neurosci.* 30, 12288–12300
- 149 Mueller, D. *et al.* (2010) Infralimbic D2 receptors are necessary for fear extinction and extinction-related tone responses. *Biol. Psychiatry* 68, 1055–1060
- 150 Gilbertson, M.W. *et al.* (2002) Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat. Neurosci.* 5, 1242–1247
- 151 Shin, L.M. and Liberzon, I. (2010) The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology* 35, 169–191
- 152 Lin, H.C. *et al.* (2008) Chronic cannabinoid administration in vivo compromises extinction of fear memory. *Learn. Mem.* 15, 876–884
- 153 Andre, J.M. *et al.* (2011) Nicotine ameliorates NMDA receptor antagonist-induced deficits in contextual fear conditioning through high-affinity nicotinic acetylcholine receptors in the hippocampus. *Neuropharmacology* 60, 617–625
- 154 Kenney, J.W. *et al.* (2010) The enhancement of contextual fear conditioning by ABT-418. *Behav. Pharmacol.* 21, 246–249
- 155 Davis, J.A. and Gould, T.J. (2007) beta2 subunit-containing nicotinic receptors mediate the enhancing effect of nicotine on trace cued fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl.)* 190, 343–352
- 156 Chess, A.C. *et al.* (2009) L-kynurenine treatment alters contextual fear conditioning and context discrimination but not cue-specific fear conditioning. *Behav. Brain Res.* 201, 325–331
- 157 Prado-Alcala, R.A. *et al.* (1994) Reversal of extinction by scopolamine. *Physiol. Behav.* 56, 27–30
- 158 Grabe, H.J. *et al.* (2009) Serotonin transporter gene (SLC6A4) promoter polymorphisms and the susceptibility to posttraumatic stress disorder in the general population. *Am. J. Psychiatry* 166, 926–933
- 159 Xie, P. *et al.* (2009) Interactive effect of stressful life events and the serotonin transporter 5-HTTLPR genotype on posttraumatic stress disorder diagnosis in 2 independent populations. *Arch. Gen. Psychiatry* 66, 1201–1209
- 160 Bryant, R.A. *et al.* (2010) Preliminary evidence of the short allele of the serotonin transporter gene predicting poor response to cognitive behavior therapy in posttraumatic stress disorder. *Biol. Psychiatry* 67, 1217–1219
- 161 Mehta, D. *et al.* (2011) Using polymorphisms in FKBP5 to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies. *Arch. Gen. Psychiatry* 68, 901–910
- 162 Attwood, B.K. *et al.* (2011) Neuropsin cleaves EphB2 in the amygdala to control anxiety. *Nature* 473, 372–375
- 163 True, W.R. *et al.* (1993) A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. *Arch. Gen. Psychiatry* 50, 257–264