An Overview of Translationally Informed Treatments for Posttraumatic Stress Disorder: Animal Models of Pavlovian Fear Conditioning to Human Clinical Trials

Supplemental Information

Additional Background Material

Pavlovian Fear Conditioning and Extinction as a Model of Fear-Related Phenotypes of PTSD

Pavlovian fear conditioning is a form of associative learning that has been used to probe fear-related phenotypes of PTSD. In this behavioral paradigm, a neutral stimulus is presented repeatedly in tandem with an aversive stimulus (unconditioned stimulus, “US”). After repeated co-presentations, the subject learns the neutral - now conditioned (“CS”) - stimulus predicts an incoming US. Consequently, the subject will exhibit fear-related behavior in response to the CS. Procedurally, Pavlovian fear conditioning is likely very similar to acquisition of traumatic memory, where individuals fearfully react (unconditioned response) to trauma (US), concurrently forming associations between the environmental context (CS) and other sensory stimuli and trauma.

Conversely, extinction involves repeated presentations of the CS so that the subject learns that the CS no longer signals an incoming US and inhibits fear-related behaviors. Extinction of conditioned fear may reflect normative fear diminishment after trauma. It is plausible that at-risk individuals exhibit poor extinction of fear responses to trauma-related cues, promoting development of PTSD. In support of this hypothesis, data suggest that individuals with PTSD exhibit impaired recall of extinction when fear conditioned with discrete cues (1-3). Extinction of cued fear also closely models exposure therapy - the recommended first-line treatment for PTSD. Procedurally, extinction is nearly identical to exposure therapy, exhibiting good face validity. During extinction, the feared object, context, or memory is repeatedly presented or

recalled in a safe environment until fear is inhibited. Moreover, extinction exhibits predictive validity, as drugs that facilitate extinction in animal models, such as D-cycloserine, have shown efficacy in some clinical studies, reducing PTSD symptoms when administered in combination with exposure therapy.

Critically, fear extinction is context-dependent, where the extinguished fear response is only observed in the context where extinction training took place and re-emerges in novel contexts. This phenomenon, referred to as “renewal”, can be used to test contextual processing deficits thought to contribute to an inability to modulate fear in individuals with PTSD (4). This hypothesis is supported by data demonstrating that PTSD patients are unable to effectively renew fear memories in a danger context compared to trauma controls (3).

While evidence suggests that individuals with PTSD exhibit poor extinction, as well as deficits in contextual processing, the extant data also demonstrate that affected individuals exhibit increased generalization, where intense fear is elicited by vaguely-related trauma cues. Pavlovian fear conditioning can also be used to model generalization. Generalization is often measured in pre-clinical animal studies by assaying fear-related behavior in response to a cue (referred to as “CS-“ or generalization stimulus) similar to the CS+, but not previously associated with a US, e.g. a 2.5 kHz versus a 10 kHz auditory tone (5-7). Similar studies have been employed in humans and demonstrate that individuals with PTSD exhibit overgeneralization (8-13).

While Pavlovian fear conditioning has been used effectively to model a number of fear-related phenotypes of PTSD, a critical caveat is whether normative fear learning is reflective of a psychiatric disorder characterized by pathological fear with evident changes in underlying neurobiology. Consequently, animal models that are hypothesized to more appropriately recapitulate PTSD are being developed and tested (14, 15). For instance, single prolonged stress (2 hour restraint stress, 20 minute forced swim, and ether anesthesia) causes deficits in fear extinction, which is thought to more thoroughly instantiate PTSD-like symptoms and the

accompanying underlying pathology (16, 17). Similarly, genetic mouse models of PTSD have been developed, including the 129S1/SvImJ (S1) transgenic mouse line, which exhibit persistent impairment of fear extinction (18). Still others have modeled PTSD-like memory impairments using hippocampal corticosterone infusions and standard Pavlovian fear conditioning (19). These models support human epidemiological data, which suggest that factors external to learning events surrounding specific trauma, including interoceptive contexts, influence development of PTSD.

Neurobiology of Pavolvian Fear Conditioning and Extinction

The neurobiology of Pavlovian fear acquisition is well characterized (Fig. S1A). Neuroimaging, lesion, and pharmacology studies across species suggest that information about the CS and US converge at the lateral and basolateral amygdala (LA and BLA, respectively) via afferents from the thalamus and cortex (20, 21). In this model, multiple pairings of the CS and the US induce synaptic plasticity at the level of the BLA (22). Subsequent activation of the central amygdala (CeA), via BLA input, elicits conditioned stimulus-elicited fear responses, including freezing, increased heart rate, and potentiated startle, by activating downstream brain areas like the hypothalamus, locus coeruleus, and other brainstem nuclei (23). Fear expression is dependent on prelimbic cortex (PL)-amygdala signaling, potentially via hippocampal modulation of the PL (4).

Alternatively, the neurobiology of extinction learning – where fear is diminished through the process of exposure to the fear-eliciting CS in the absence of the aversive US - is incompletely understood (Fig. S1B). Data suggests extinction learning is a new learning process that proceeds through multiple mechanisms to suppress, rather than erase, existing aversive memories (24). Dynamic changes in gephyrin mRNA and $GABA_A$ receptor binding indicate an increase in amygdala GABAergic transmission with extinction (25). *In vivo* electrophysiology studies reveal a subset of excitatory projection neurons in the BLA exhibit increased firing rates

during extinction (26). These “extinction neurons” are responsive to the CS during extinction, but not during fear conditioning (26). Furthermore, a number of studies implicate the mPFC, in particular the infralimbic cortex (IL), in extinction recall, with data suggesting that the IL inhibits CeA ouput, potentially via hippocampal activation of the IL (4, 27-29). IL-dependent potentiation of BLA inputs to GABAergic ITC neurons increases inhibition of the CeA during extinction, decreasing conditioned fear responses (30, 31). The mPFC is similarly implicated in renewal. Renewal is thought to depend on hippocampal-controlled gating of amygdala responses via direct projections to the amygdala and/or indirect projections via mPFC (4). Additionally, regions with an established role in fear learning are critical for fear generalization (6, 8, 32-35).

Importantly, individuals with PTSD exhibit amygdala hyperactivity and mPFC hypoactivity, consistent with neurobiological data from fear conditioning and extinction experiments conducted across animals and humans (36, 37). Specifically, changes in coupling between the amygdala and mPFC may underlie emotion dysregulation in PTSD, where reduced mPFC-driven inhibition allows amygdala hyperactivity (38). Reduced hippocampal volume has also been associated with PTSD, potentially contributing to observed contextual processing deficits (36). For more thorough review examining the neurobiological correlates of PTSD, see (36, 37).

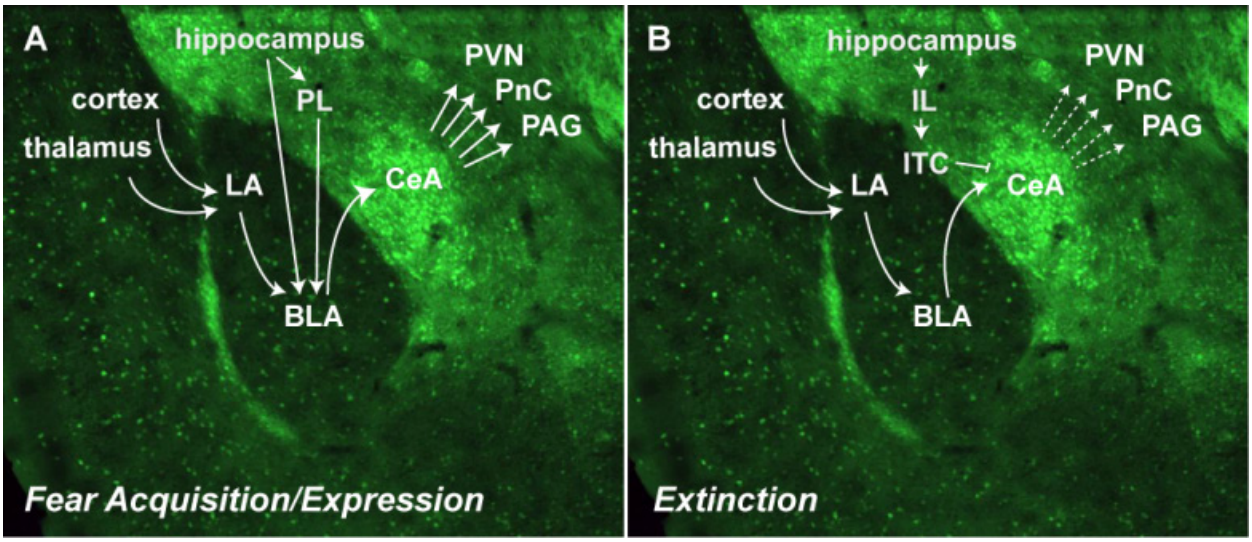

Figure S1. Neurobiology of associative fear learning. Expression of enhanced green-fluorescent protein (GFP) in these photomicrographs is driven by the interneuron-specific driver Dlx5/6-Cre. During fear conditioning, information about the conditioned stimulus (CS) and unconditioned stimulus (US) converge at the lateral and basolateral amygdala (LA and BLA, respectively) via afferent neural projections from the thalamus and cortex. Multiple co-presentations of the CS and US induce synaptic plasticity at the level of the BLA. Subsequent activation of the central amygdala (CeA), via BLA input, elicits conditioned stimulus-elicited fear responses (CR), including freezing, increased heart rate, and potentiated startle, by activating downstream brain areas like the hypothalamus, pontine reticular nucleus (PnC), periaqueductal gray (PAG), and other brainstem nuclei. Prelimbic cortex (PL)-amygdala signaling, potentially via hippocampal modulation of the PL, is critical for fear expression, and has been similarly implicated in renewal, where conditioned fear responses re-emerge outside of the extinction context. Renewal of fear is also dependent on direct projections from the hippocampus to the amygdala. B) The neurobiology of cued fear extinction is less clear. Activation of GABAergic intercalated cells by mPFC, specifically the infralimbic cortex (IL), is thought to dampen CeA output, inhibiting the expression of fear-related behaviors. Hippocampal modulation of IL is thought to drive, in part, IL-mediated inhibition of CeA.

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